JUN 24 1922

BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

Vol. XXXIII—No. 376] BALTIMORE, JUNE, 1922 [Price, 50 Cents

CONTENTS

PAGE

The Evolution of Human Races in the Light of the Hormone Theory. Racial Status and Form of Body. By Prof. Sir Arthur Keith 195

The Relation of Nutrition to Tooth Development and Tooth Preservation. I. A Preliminary Study of Gross Maxillary and Dental Defects in Two Hundred and Twenty Rats on Defective and Deficient Diets. (Illustrated.) By E. V. McCollum, Nina Simmonds, Ethel M. Kinney and Clarence J. Grieves 202

Studies on Experimental Rickets. XX. The Effects of Strontium Administration on the Histological Structure of the Growing Bones. (Illustrated.) By P. G. Shipley, E. A. Park, E. V. McCollum, Nina Simmonds and Ethel May Kinney . . 216

PAGE

Carcinoma of the Cervix Uteri. A Very Early Case. (Illustrated.) By Karl H. Martzloff 221

The Capillaries of the Bone Marrow of the Adult Pigeon. (Illustrated.) By Charles A. Doan 222

The Effect of Slight Increase of Temperature on the Bacteriostatic Power of Gentian Violet. (Illustrated.) By John W. Churchman 227

Studies on Experimental Rickets. XXI. An Experimental Demonstration of the Existence of a Vitamin Which Promotes Calcium Deposition. By E. V. McCollum, Nina Simmonds, J. Ernestine Becker and P. G. Shipley 229

Entered as Second-Class Matter at the Baltimore, Maryland, Postoffice.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

THE EVOLUTION OF HUMAN RACES IN THE LIGHT OF THE HORMONE THEORY*

RACIAL STATUS AND FORM OF BODY

By Prof. Sir Arthur Keith

(Conservator of the Museum and Hunterian Professor, Royal College of Surgeons, London, Eng.)

Herter Lecture No. 2.

Giantism and Acromegaly.—Giants are usually the subjects of acromegaly as well as of overgrowth of trunk and limbs. There have been authentic cases of giantism which were free from the usual manifestations of acromegaly. There are also cases, certainly few in number, of adolescents who were the subjects of acromegaly and yet showed no increase in stature. From such instances one infers that, although giantism and acromegaly may be present in the same subjects, they represent disturbances of two correlated yet distinct growth mechanisms. During the years of growth there must be some ever present process at work controlling the growth of the correlated functional systems of the body. Since the pituitary is enlarged in giants, who are free from acromegalic traits, we may infer that this mechanism is also represented in the pituitary—in part at least—for other centres of growth also influence stature. Giantism represents a disorder of the hormone mechanism presiding over, not the reactional, but the normal growth of parts.

Localized Acromegalies.—In seeking to unravel the nature of acromegaly one is at first tempted to suppose that the condition is merely the result of the pituitary throwing increased doses of a growth-elixir or hormone, into the circulatory blood. A study of cases of localized overgrowth or partial acromegaly shows that the expla-

* Abstract of Lectures Nos. 2 and 3 of the thirteenth course of lectures under the Herter Foundation, delivered before the Johns Hopkins University, October 6, 1921.

nation cannot be so simple. The most instructive cases of this kind are those seen in children in whom there is a marked unilateral hypertrophy of the apparatus of mastication. One side of the face is acromegalic, the other is normal. A minute examination of such cases shows that the overgrowth is sharply limited to the parts concerned in mastication—to one half of the mandible, to the corresponding maxilla and palate, and to the bones which give attachment to the corresponding muscles of mastication. The tympanic plate is involved in the overgrowth, showing that it is part of the apparatus of mastication. The disorder must commence in fœtal life, for the teeth of the affected side are much larger than on the normal side. There is no evidence as yet that there is an anatomical change in the pituitary glands in such cases, but as we have here an undoubted manifestation of localized acromegaly, it is legitimate to infer that we must be dealing with a disturbance of the same hormone-machinery that lies behind generalized acromegaly. In such cases we see the anatomical elements of a single functional unit picked out. In generalized acromegaly all functional units are affected.

The mechanism of Growth Hormones.—These localized cases of acromegaly show that growth is not merely the result of the presence of an activating substance thrown into the general circulation by one or by a number of glands of internal secretion. A local as well as a general mechanism must be concerned. In the case of *functional* hormones, such as secretin and adrenalin, and also in the case of *growth* hormones formed within the testicle, we see that such substances, although offered to all the tissues of the body are selected and accepted only at certain definite points or regions. It is not difficult to conceive that one of these local receptive mechanisms might be heightened in avidity or susceptibility and thus give rise to a local hypertrophy. Whatever the nature of the central and peripheral growth mechanisms may prove to be, there can be no doubt that they are arranged on a physiological basis—one which bends a collection of anatomical elements to serve definite functional ends. It is this complex dual mechanism which is inherited from generation to generation and which fashions mankind into different functional types and races.

Examples of the Pituitary Mechanism at Work in the Evolution of Types.—Up to this point I have endeavoured through a study of acromegaly to unravel the complex mechanism which regulates the growth of the human body, concentrating attention more especially upon the apparatus of mastication. To exemplify the manner in which the pituitary growth mechanism produces its effects, no finer example can be chosen than man's nearest relative—the gorilla. He is the greatest and strongest member of the zoological group to which man also belongs—the Higher Primates. The skull of the baby gorilla is smooth and rounded like that of a child; after infancy there sets in a series of growth changes which transform the whole appearance of the individual; the masticatory apparatus reaches a supreme degree of development; the temporal lines rise up to form the great sagittal and lambdoidal crests of bone, the lower part of the frontal bone is extended forwards until it forms a great ledge-like bar of bone above the orbits; the jaws and face become massive. Behind this transformation must lie the same pituitary growth mechanism which is revealed in the acromegalic. In the chimpanzee the growth machinery comes to a standstill at a stage reached in the juvenile gorilla; in man it is arrested at a stage represented in the childhood of the gorilla. In Neanderthal man we also see the pituitary mechanism vigorously at work. The ape-like supraorbital ridge, the massive jaws, and the wide expanse of the nuchal platform are evidences of this influence. But it is among peoples of the Caucasian or European type that we may best mark the peculiar effects of this mechanism among modern races of mankind. The homeland of this type extends from the western confines of India to the Atlantic shores of Europe. So far as the facial features are concerned, a long narrow prominent nose, a prominent chin with a tendency to elongation and narrowing of the face are the chief racial marks of this type. These are features which almost invariably become greatly exaggerated in the acromegalic. In the shaping of the European type, the pituitary mechanism has had much to do. In shaping the racial characters of the European type the pituitary influence seems to have dominated the other centres of hormone-production; in the Mongolian and Negro types other centres, such as the thyroid and adrenals seem to have gained in potency.

The Origin of new racial characters and types.—Although I have by no means exhausted the anthropological lessons to be derived from the study of pituitary disorders—such as are seen in giantism, Fröhlich's disease (dystrophia adiposo-genitalis), dwarfism, infantilism, progeria—it is important, before we proceed further, to study the manner in which new racial characters come into existence—particularly those relating to the colour of skin and hair and to the nakedness of the human body. In the Museum of the Royal College of Surgeons there has been exhibited since the conservatorship of Sir Richard Owen, the pregnant uterus of a chimpanzee, dissected so as to expose a fœtus in the seventh month of development. Prof. Louis Bolk of Amsterdam has a similar specimen. Independently he and I have realized the important bearing which such specimens have on the theory of hormone evolution.* So human are the uterus and fœtus in appearance that a superficial observer might well believe he had before him the womb and fruit of a woman. The skin of the body is nude, except for the

* "The Part Played by the Endocrine Glands in the Evolution of Man." Lancet, 1921, [illegible] 588.

presence of a fine lanugo; on the other hand, the scalp is covered with hair of a brown colour. The distribution of hair is human—not anthropoid. The skin is scarcely pigmented; it is ashen grey in colour; at a corresponding stage the negro fœtus is not dark skinned. Prof. Bolk draws the conclusion, and I agree with him, that man has come by his naked skin and the European by his white colour by inheriting a fœtal condition from his anthropoid ancestry. A transient fœtal condition of the anthropoid has become fixed permanently in the human adult. We have seen that in his masticatory apparatus and in his nuchal fixation, man retains stages seen in the childhood of anthropoid apes. Man's new characters have first been elaborated within the uterus of the ape; later these fœtal characters have been incorporated in the make-up of the human adult.

Up to this point Prof. Bolk and I proceed together. But we must go further and ask ourselves the question, How do these fœtal characters arise in the anthropoid womb? I have laid stress upon the fact that the pituitary mechanism—the whole mechanism of growth hormones—has been elaborated and organized on a functional or physiological basis. This was made apparent when dealing with the effects of the pituitary mechanism in fashioning the apparatus of mastication. Nor do we make any extravagant demands on our imagination when we proceed to suppose that such hormone mechanisms are constituted and elaborated during the fœtal life, in the same way as the mechanisms of instinct and of reflex action are given a concrete representation in the nervous system. The nerve-machinery of an automatic action is inherited. One may hazard the presumption that this also may be true of the hormone machinery, which presides over the functional fashioning of the body. The long developmental intra-uterine period of higher vertebrates, particularly of the higher primates, gives the hormone system the shelter necessary for working out its effects in the fœtal body and the opportunity of attempting fresh experiments. It is under the influence of this automatic hormone mechanism that new characters are elaborated and fresh experiments launched during the developmental stages of life. Darwin's Law of Natural Selection tests these experiments, accepting the successes, and rejecting the failures.

Hair and Skin Characters.—Colour is one of the principal marks of race, but in explaining why we should find all shades from the deep black of the negro to the fair skin of the Norseman, we are handicapped by our ignorance of the part played by pigment in the economy of the body. The facts at our disposal are these: When the medullary parts of the adrenal bodies are injured or destroyed by disease, the skin becomes pigmented—as Thomas Addison noted in 1855. John Hunter inferred that the original colour of man's skin was black and all the facts we have gathered since his time favour this view. The gorilla is the negro amongst anthropoids, he is deeply pigmented at birth. The chimpanzee darkens after birth, some races early, others later. In the orang the hair is red but the skin has a slaty hue. We have already seen that the fœtus of man, as of anthropoid, is free from pigment.

There is thus a relationship between pigmentation and these important centres of hormone production—the adrenal glands. We obtain some light on our problem when we remember that the adrenals are closely connected with the important function of maintaining the body at a temperature—under all extremes of heat or cold—of approximately 98.4° Fahr. The regulation of the blood supply to the skin, the secretion of sweat, and the development of subcutaneous fat, are the important means by which this end is secured. The development of hair on the body, the amount and distribution of pigment and fat must also be accessory circumstances in the regulation of temperature. We have seen how anatomical elements are combined into functional units by means of a hormone mechanism and I infer that future research will reveal a growth mechanism which presides over the parts concerned in the regulation of temperature and that skin-pigment will be found to be included in this system. The mechanism must be a complex one in which pituitary and thyroid are also involved, for there is no doubt that disorders of the pituitary are followed by definite changes in hair and skin; thyroid disorders also give rise to changes, but they are of another kind. From these facts we see that the skin is dominated by a hormone mechanism in which adrenal, thyroid and pituitary are combined—a mechanism of a complex nature.

Thyroid Effects.—On entering into a discussion on the effects produced by thyroid substances in shaping the racial characteristics of mankind, it is necessary to recall some of the statements made regarding the nature of the machinery lying behind the growth changes produced by hormones. I am regarding the glands of internal secretion as centres for the elaboration and distribution of hormones and believe that the organs and tissues of the body are endowed with special affinities for the hormones in circulation and that very probably these local centres have also a means of making their needs known at the centres of hormone production. In dealing with pituitary effects I have proceeded on the belief that we are witnessing positive results—an increased action of the pituitary producing increased activity in definite peripheral sites of growth. Thyroid substances can produce acceleration of growth; this is strikingly shown by the manner in which extracts of the gland can increase the growth of cretinoid children and hasten the metamorphosis of tadpoles. I presume that thyroid and pituitary growth hormones utilize the same local or peripheral mechanism in producing their effects.

Cretinism.—It is, however, not the positive or exaggerated action of the thyroid, but its minus or defective one which brings out the part played by this gland in the differentiation of races. In cretins, growth of all the tissues of the body is not only retarded but positively distorted. The effects are best seen in the cartilaginous growth discs of long bones. The cartilage cells multiply slowly and irregularly; osteoblasts cease to have the power to invade and overcome them. The cartilaginous base of the skull suffers in this way; the root of the nose becomes drawn in and widened; the nose itself, which is built over a foundation of cartilage, is wide, short and not prominent. The face, which in acromegaly is long and narrow and wedge-shaped, is short, wide and flat in the cretin. The skin is dry, the hair scanty, and the subcutaneous tissue thickened. These are the results of a gross deficiency of thyroid substances. When thyroid extracts are administered, the characteristics previously produced disappear and are replaced by the normal. We have in cretinism positive evidence that the thyroid has to do with regulation of stature and the determination of characters of face, hair and skin—all being marks employed by anthropologists in the classification of races.

Mongolism and Achondroplasia.—Cretinism is a result of a gross deficiency of thyroid substances. There are at least two disorders of growth—mongolism and achondroplasia—which one has reason to attribute to a defective or altered action of thyroid substances. It is true that the exhibition of ordinary thyroid extracts has no effect upon such cases, nor has any one succeeded in producing these conditions experimentally in animals, yet there are clinical manifestations and structural alterations in both of these disorders, which are also seen in cretinism, and for this reason I feel justified in attributing both mongolism and achondroplasia to disturbances in the complex effect exerted by thyroid substance on the growth and differentiation of the body. Dr. Langdon Down, in 1866, recognized that a certain class of imbecile was characterized by traits which recalled those seen in the faces of Mongolian peoples. My friend, Dr. Francis Crookshank has explained the appearance of such traits as a harkback to a Mongolian ancestry. Not only is there a lack of evidence that a Mongolian people has at any time populated Europe, but there is circumstantial evidence that the Mongolian type of humanity is one of the most recently evolved of racial forms. The theory which best explains all the facts is to suppose that Mongolian features arise under a peculiar or altered action of the growth mechanisms centered in the thyroid gland.

It is not necessary to recall the bodily characteristics of achondroplasia or "bulldogism"—for the disturbance of growth is that seen in all varieties of the bulldog breed. The trunk is normal in size but all segments of the limbs, including shoulder and pelvic girdles, are retarded and distorted in growth. In particular, the base of the skull suffers;* the cerebellar fossa and the area of nuchal fixation are reduced in size and greatly altered in shape; the root of the nose becomes drawn in between the eyes, and broadened just as in cretins. The facial bones laid down over the nasal capsule are retarded in growth, so that the anterior part of the maxilla is drawn up, producing a pug-face. As in dachshunds, the limbs may be affected while the base of the skull and nose develop normally. The disorder occurs in all races of mankind and in varying degrees of severity. Darwin described a bulldog breed of cattle from the Argentine. Dr. Seligman found that the cretinoid or bulldog calves of Kerry cows had disordered thyroids. Dr. Douglas Symmers observed that in a proportion of still-born achondroplastic children there was a structural disorder of the thyroid gland. In achondroplasia we are clearly dealing with a disorder of a growth mechanism which is widely distributed in the animal kingdom and the evidence points to this mechanism having its centre in the thyroid gland.

Now Mongolian peoples are characterized by limbs which are short in comparison with their trunk length. The nasal region between the eyes is wide, flat and frequently sunken in typical Mongols. The flattening of the root of the nose is even better seen in the true Negro type. In the Mongol, as in the cretin, eyebrow ridges are poorly developed or absent. It is not only amongst human races that these supposed thyroid effects can be traced. The orang is the bulldog or Mongol amongst anthropoid apes; he is pug-faced, the nasal region of his face being greatly reduced and drawn in, the supraorbital ridges are developed to only a minor extent—especially when contrasted with their development in the gorilla. With this conformation goes a silent and rather morose temperament. The orang differs in colour from other anthropoid apes just as the Mongolian colouring contrasts with that seen in the negro or European. The late Prof. H. Klaatsch was struck by the points of resemblance which linked the Mongolian and orang types and sought to explain the similarity by supposing that the Mongol and orang had a common ancestry. The explanation I offer is that both have inherited a common mechanism of growth-hormones and they are superficially alike because, in both, the thyroid effects of this mechanism have become dominant.

Dwarf-Races and Stature.—No anthropological problem has been more discussed than the position to be assigned to pygmy or dwarf varieties in the scale of human evolution. In finding the proper solution to this problem, it has to be remembered that all living dwarf breeds are members of the negro race and that each dwarf variety has many resemblances to the breed of negro existing in the same region. The central African pygmies are dwarf

* Quart. Journ. Med., 1912, Vol. 5, p.157.

forms of the true negro; the Bushman is a dwarf form of the Hottentot breed and the pygmies of the Far East are varieties of the Eastern Negroids. From their characters and distribution one infers that pygmy peoples have been produced locally from normal members of the negro race, just as dwarf races of horses, dogs and cattle have arisen locally from breeds of normal size. It is also worthy of note that the Dinkas and Shilluks of the White Nile are amongst the slenderest and tallest examples of mankind. Stature is a highly variable factor in the negro race. When we attempt to distentangle the exact nature of the growth mechanism which leads to the production of dwarf races, we become keenly alive to the complexity of the problem and to a need for further knowledge. All tissues of the dwarf body are fashioned on a miniature scale, but we can best realize what happens by concentrating our attention on the epiphyseal lines or growth discs of long bones which are so directly related to stature. Epiphyseal lines represent local growth mechanisms, activated and controlled by substances thrown into the circulation at centres of hormone production. Pituitary substances play on these sites of growth; we explain giantism by an oversupply, and infantilism, such as is seen when the pituitary is compressed by a cyst, by an under-supply. The sexual glands can also influence the rate of growth at epiphyseal lines, but they exert their influence not directly on the cells of the epiphyseal lines but indirectly through the cortical part of the adrenals. A fuller knowledge of the part played by the growth hormones of the thyroid is likely to give us an explanation of the origin of dwarf breeds.

The Hottentot Type.—The wide, flat interocular field of the typical Mongol is to be regarded, so I think, as an effect of thyroid action; the same inference must also hold good in the case of the Negro. Now, in the most peculiar breed of existing Negroes—the Hottentots of South Africa—the interocular field is particularly wide and flat; the bony skeleton of the nose is reduced; the supra-orbital ridges are poorly developed. Many anthropologists have noted Mongolian traits in the Hottentot face, as well as the yellowish tint of their skin. These traits are also to be noted in the face and skin of the Bushman. If we admit that there is a tendency for the thyroid mechanism to become dominant in the Negro type, then we can explain the appearance of Mongolian traits in the Hottentot—undoubtedly a member of the Negro race. No other theory can give a satisfactory explanation of the racial features and of the distribution of the varieties of the Negro-type.

Dystrophia adiposo-genitalis.—Having touched on the Mongolian traits of the Hottentot, it is convenient at this point to discuss another anthropological character by which the females of this racial type are marked—a tendency to the accumulation of fat on the buttocks and outer aspect of the thighs. This peculiar character occurred in certain peoples who lived in Europe towards the close of the ice age, as we know from discoveries of statuettes and drawings of that distant period. These fat-buttocked women were of the European type, although their skulls do show some negroid traits. The changes which occur in modern women at the menopause help to throw some light on the physiological machinery underlying the appearance of such a feature as a race mark, for, as is well known, atrophy of the ovaries is frequently followed by the deposition of fat, particularly in the region below the waist. The same result frequently follows removal of the ovaries or testes. In the growth disorder first described by Fröhlich—*dystrophia adiposo-genitalis*—there is a like tendency to the subcutaneous accumulation of fat, but in such instances deposition follows enlargement of the pituitary gland and an atrophy of the sexual system. On our present evidence, we cannot say whether the change in the pituitary or in the sex glands is the primary one—very probably it will prove to be the latter—but we may safely attribute the deposition of fat and the assumption of a female configuration of body as a direct result of the sexual atrophy. In Hottentot women the physiological process underlying the localized deposition of fat comes into play independently of any loss of sexual function and yet upon the evidence of pathology we must suppose that this process is controlled through a hormone mechanism centred in the sex glands. There is ample evidence to show that a close functional relationship exists between the centres of hormone production—the adrenals, pituitary, pineal and thyroid glands. The sex glands have apparently the power, by means of ovarian or testicular hormones, to influence and set into operation the growth-controlling machinery situated in the glands of internal secretion. All other evidence points to the influence of the sex glands as being one which is exerted, not directly on the tissues of the body, but through the intermediation of other centres of hormone production. The deposition and absorption of fat we must regard as controlled by an interaction between adrenals and thyroid—the glands concerned in regulating the metabolic rate and temperature of the body. The regional deposition of fat recalls, as do localized acromegalies or overgrowths, the need for postulating a local as well as a central machinery in our explanation of the efforts of growth-hormones. It is worthy of remark that the profuse wrinkling of the face, so commonly seen in adult Bushmen and Hottentots, is also present in eunuchs and eunuchoid beings. In these conditions the texture and elasticity of the skin are also affected.

The Nilotic Type of Negro.—Before leaving the Negro type, from which I have selected the foregoing instances to illustrate the application of the hormone theory to the explanation of racial features, there is a further example I may be allowed to cite. The Negro tribes along the White Nile are slender and tall, their height being largely due to length of limb. Their extremities, in opposition to

those of the Mongolian type, are long in comparison to length of trunk. Now castration, as is well known, results in a delayed closure of the epiphyseal lines. Eunuchs are taller and longer limbed than are normal men belonging to the same place and race. At puberty, when the influence of the sex glands begins to play on the other hormone centres, the body loses the slenderness of boyhood and girlhood; in the Nilotic negro type the slenderness of youth is retained in adults—a further example of the evolution of a new racial type by the fixation of parts at an immature phase. The sex glands in this type appear to reach maturity without setting in motion the machinery which expands the framework of the body and thus undoes the slenderness of youth. It is an example similar to that cited in the Hottentot women—where menopause characters appeared without impairment of the sexual function of the ovaries.

Herter Lecture No. 3.

Sex-Glands and Adrenals.—From remote times mankind has known that removal of the sex glands completely alters the characters of body and mind of the human as well as of the animal body. What is new in this department of knowledge is the hormone theory—an explanation of how the effects of castration are brought about. Until the postulation of the theory of hormones by Starling in 1904, medical men had supposed that the parts and systems of the body were provided with only one means of intercommunication—a nervous or telegraphic system. By 1904 knowledge had reached a point where it could be seen that there was an infinitely older system of intercommunication—a postal or hormonic system—which as regards the growth of the body is very much more important than the nervous system.

Tumours of the Adrenal Cortex.—The important rôle of the pituitary gland as a regulator of growth was an accidental discovery. Clinicians and pathologists discovered that disorderly overgrowth of the body was accompanied by an enlarged and adenomatous condition of this gland. Medical science benefitted by Nature's pathological experiment. In exactly the same way we have learned that the cortex of the adrenals is an important centre of growth-control. This glandular tissue is also liable to become the site of a localized overgrowth or an adenoma in the young and is accompanied by a very definite series of growth changes in the body. Recently my friend Prof. E. E. Glyn * of Liverpool has rendered an important service by tabulating the data relating to cases of tumour of the adrenal cortex, adding several instances which have come under his own observation. I had an opportunity of seeing the classical case described by Bulloch and Sequeira in 1903. The occurrence of such tumours in young boys is accompanied by a premature development of all the bodily changes which should not take place until the age of puberty. A boy of five years of age will become sturdy and thick-set—an infant Hercules; his voice will break; hair will grow on the face and pubes; his penis becomes developed; the testes become active. The adrenal cortex presides over the development of all of the structural parts which are fashioned into "secondary sexual characters"—the name given to them by John Hunter. Cases are recorded in which these changes have set in soon after birth—pubertal changes masking the features of babyhood. In female children or in young women, cortical tumours of the adrenal produce a different effect; they tend to bring out the sexual characters of the male. With the surgical removal of such a tumour, the normal female configuration of body, voice and of mind becomes restored, the adventitious male characters disappearing.†

On the evidence of embryology alone one would suspect that there must be a close functional relationship between the sexual glands and the adrenal cortex; both are differentiated at the same site and apparently from a common embryological basis. One infers that it is through the adrenal cortex that the sex glands bring about the complex series of growth changes which transform the human body at puberty. Through the pituitary mechanism these same glands can play upon the general growth of the body systems.

Tumours of the pineal gland may be accompanied by a series of growth changes almost identical in nature and scope to those following tumours of the adrenal cortex. How such a function has come to be resident in the pineal is an enigma at present—one which future research will probably clear up.‡

The Rôle of the Adrenals in Race Differentiation.—It is scarcely necessary to recall the fact that secondary sexual characters, which are apparently controlled by hormones elaborated in the adrenal cortex, are employed by anthropologists as marks for differentiating one race of mankind from another. The beard and hairiness of body which characterize the European or Caucasian type of man, and also, to a lesser extent, the Australoid type—

* See Keith. Journ. of Anatomy and Physiology. 1910, Vol. 44, p. 251; *ibid.* 1913, Vol. 47.

† Prof. T. R. Elliott (personal communication).

On my arrival in New York I visited Prof. Stockard's Laboratory, Cornell University, and found that he had also arrived at many of the conclusions which are given expression to in these lectures. Prof. Stockard has applied the theory of hormones to explain the various breeds of dogs as well as of human beings.

‡ (1) For recent literature on the pineal gland, see Krabbe: Meddel. fra Rigshosp. Borneafdeling. 1917. No. 48.

the most primitive of existing races—I look upon as an ancient [redacted] characteristic of humanity. In Mongolian and in Negro types the tendency is to a hairless face and body. In this respect these types are more highly evolved than the European. The immature stage, seen in the European youth, is tending to become fixed as the adult stage in the Mongol and Negro. The growth mechanism which determines the woolly hair and thick everted lips of the negro we do not know, but are justified in regarding both of these features as non-anthropoid and of recent origin. Large labia majora, on the other hand, are characteristic of the European type. Prof. Bolk has pointed out that these structures are relatively large in fœtal stages of ape and man and that the European has acquired this feature by a fœtal becoming an adult character.

The Periods of The Life-Span.—All the evidence at our disposal points to the evolution of higher human races as having been accompanied by a lengthening of the various growth periods which make up man's normal span of life. The more civilized races of man have longer lives than the primitive races and the primitive races than the great anthropoid apes. It is quite apparent, from what occurs in children suffering from tumours of the adrenal cortex, that Nature has a hormone mechanism at her disposal for accelerating or retarding the maturing of the body. The action of the adrenal cortex must be connected with the regulation of the periods of growth and with the span of life. We have further examples of an interference with the normal maturation of the body in the group of cases at present described under the generic term—infantilism. There are forms such as that illustrated by a specimen in the Museum of the Royal College of Surgeons—the skeleton of Crachami—a girl, 8½ years of age, but in size and in many other features similar to a baby only a few months old. Dr. Hastings Gilford has distinguished this form as a fœtal type of "infantilism." Then there is a type illustrated by such cases as that of Jeffrey Hudson who died at 63, and Bornolaski who died at the age of 98. In such cases the span of childhood covers the whole period of life. They may become sexually potent and yet retain a childish size and appearance, their epiphyseal lines remaining open late in life. Again there are those remarkable cases of infantilism to which Hastings Gilford has given the name of Progeria. Such individuals assume an aged appearance while still in their girlhood or boyhood. The exact nature of the growth disorder in these cases is not known, but it is probable that both pituitary and adrenal mechanisms are involved. Compression or atrophy of the pituitary does arrest the growth of the body—produces a condition of infantilism. In the case of progeria that I have examined, the pituitary fossa is relatively small and the neck of the gland is surrounded by a bony ring formed out of the clinoid processes. The atheromatous condition of the arteries, and the premature appearance of senility suggest that the adrenals may also be involved. The evidence is sufficient to awaken an interest in the rôle played by hormones in regulating the length of life's span.

Conclusion

I am well aware of the imperfect nature of the evidence on which I have based a plea for regarding hormones as the agencies which control growth and evolution. Before coming to a conclusion as to whether a *prima facie* case has been made out, I would beg the reader to look narrowly at the position in which all who believe in the evolution of living things are now placed. Darwin gave the law of Evolution an abiding place in Biology; his great difficulty was to explain how a multitude of anatomical elements became simultaneously modified to serve a single definite functional purpose. A study of the growth disorders reveals the fact that the hormone systems, centered in the pituitary and adrenal glands are organized on a functional basis. Hormone systems represent automatic growth mechanism which, like all living qualities, are hereditary and variable. Hormones represent the elements of an automatic system for the control of growth. For this reason new characters do not appear at the end of a developmental stage but early in the growth of the fœtus. New characters appear first *in utero;* later they become fixed as a new character in the adult stage. The hormone growth machinery is just such a one as Darwin was in search of. He propounded the elaborate theory of Pangenesis as a substitute.

In these lectures I have been applying the theory to problems relating to the origin and evolution of human races. But the day is certainly coming when it will be made applicable to the realms of Zoology and Botany and provide a real scientific basis for these branches of knowledge.

From the experiments carried out in India by Lt. Col. Robert McCarrison it now seems possible that the substances named vitamines may influence and alter the growth mechanisms of the animal body.

Lastly, from the study of English human remains, representing samples of the inhabitants who have lived in that land at successive periods during the last 4000 years, there is a convincing body of evidence that structural changes are taking place in the jaws, palates and faces of a large proportion of the present population of England. A full knowledge of the hormone system of the human body is likely to reveal not only the cause of these structural changes but also the steps which may have to be taken to combat them.

THE RELATION OF NUTRITION TO TOOTH DEVELOPMENT AND TOOTH PRESERVATION. I.

A PRELIMINARY STUDY OF GROSS MAXILLARY AND DENTAL DEFECTS IN TWO HUNDRED AND TWENTY RATS ON DEFECTIVE AND DEFICIENT DIETS

By E. V. McCollum, Nina Simmonds,
and Ethel M. Kinney
(From the Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore)
and
Clarence J. Grieves
(From the Department of Pediatrics, Johns Hopkins University)

The earliest conception of the causes of oral and dental disease may be divided into the systemic or internal and interstitial, and the external or environmental factors.

Hippocrates taught that dental caries was due to "stagnation of depraved juices in jaws and teeth," aggravated by accumulation of food debris.

Galen believed that disturbance of nutrition produced vicious humors of the blood and caused teeth to decay.

John Hunter maintained that teeth were destroyed by internal inflammation and not by external influences.[1]

The more modern etiological hypotheses may be divided into the traumatic, inflammatory, infectious, toxic, chemical, chemico-micro-organic, and nutritional.[2, 3]

Dental defects were conceived to be caused externally by trauma or by decalcification of enamel from acid foods, drinks, and medicines. After the discovery of fermentation, it was believed that micro-organisms, which converted starches and sugars into acids, existed in the mouth and decalcified enamel. Other types of bacteria were said to invade and decay dentin by both fermentative and putrefactive processes.[4]

The saliva was thought to be acid from acid foods or from acid-forming bacteria. Its acidity was also regarded as an expression of lowered blood alkalinity. Inorganic waste in the saliva, which was deposited gingivally, was known to form salivary calculus. This caused irritation of the gums and was said to induce gingival disease.

This conception of local damage by local secretions was applied to solve the etiology of dental erosion. The soluble acid calcium and sodium salts, abnormally excreted by the oral mucous glands, were believed to erode cervical enamel.

The various phases of gingival disease culminating in pyorrhea were said to result from external causes, acting from without inward; the theories of "traumatic occlusion" and of specific infectious invasion may be mentioned as examples. But many of the gingivitides were observed to have their origin in the organism itself and to progress from within outward; examples of this type resulted from scurvy and from massive doses of mercury.

Gingivitis was regarded as the result of "auto-intoxication," of "excess of uric acid," and of toxic absorption from the gastro-intestinal tract.[2] But essentially the idea was that of deranged metabolism, "suboxidation," or defective elimination, rather than that of an improperly constituted diet.

The use of bread made of decorticated and degerminated flour was regarded as one of the causes of dental caries. Upon such a diet it was stated that expectant mothers suffered much from dental caries and the child's teeth were so poorly calcified as to be subject to early decay.

Proprietary infant foods were believed to produce enamel hypoplasia, defective dentin and general dental dystrophy.

It was claimed that an excessive carbohydrate diet produced caries in both adults and children and an excess in protein was thought to produce pyorrhea in adults.[3]

The endocrine glands, particularly the parathyroid and pituitary, were indicted for causing maxillary dystrophy, malocclusion and dental disease, presumably because of their apparent relation to growth and to calcium and sugar metabolism. The oral conditions found in cretins and acromegalics were cited as examples.

It had long been assumed that dental caries was caused by excessive sugar in the diet. The incidence of caries was said to develop in the same ratio as sugar consumption. It was supposed to increase the blood sugar which, it was claimed, furnished a fermentable medium for bacteria in the dentinal tubuli. The salivary glands were said simultaneously to excrete glycogen, which fermented on enamel surfaces. So, both locally and systemically, sugar was supposed to attack the teeth.[2, 3, 4]

The literature on scurvy and rickets has been quoted to show that deficiencies in the vitamins, fat-soluble A and water-soluble B, caused caries of bones and teeth, and pyorrhea.

The statement that "a clean tooth never decays" was used in oral hygiene propaganda and marked results were reported from the practice of oral prophylaxis by patient and operator. Cleanliness is a relative condition and it is now known from a study of those who sedulously

practiced oral cleanliness that caries and gingival disease recur in the so-called "clean mouth."

From this brief etiological review, which is necessary if this report is to be understood, we can only conclude that oral and dental diseases arise from internal, or systemic, and external, or environmental, causes, or a combination of such causes.

There is a definite relation of oral types: the temporomandibular articulation, maxillæ and occlusion, the tooth form, cusps, roots and attachments, to the form of the gastro-intestinal tract and to food habits.[5] These must be considered in selecting animals for oral study. The highly specialized and persistently growing molars of the guinea-pig and its equally specialized gastro-intestinal tract are not comparable to those of man, nor is the guinea-pig, with its limited diet of roots and grasses, suited to the study of the omnivorous human diet.

The dog's salivary and gastro-intestinal functions are well known, while comparatively little is known of these functions in the rat. The dog's denture is large and accessible and presents few of the histologic difficulties found in the study of the small molars of the rat. But if dogs are allowed to run, they may obtain supplementary food, thereby disturbing the experimental conditions. If confined, they may develop nutritional disturbances, deformity and disease. The rat thrives under laboratory conditions and is, therefore, the animal of choice for studies of nutrition.

"The rodents include by far the greatest number (nine hundred) and have the widest distribution of any order of terrestrial mammals." Of the sub-family, Muridæ, which included M. Norvegicus, (used in these studies), it is said "this genus is the largest in the whole mammalian class, comprising not less than one hundred and thirty species."[6] Such preponderance is founded in great fertility and adaptability, especially to nutritional environment, one factor of which (the rodent denture) is of advantage in obtaining and masticating food.

A composite rodent dental formula consists of incisors $\frac{1}{1}$, canines $\frac{0}{0}$, premolars $\frac{0}{0}$, molars $\frac{3}{3}$, bilaterally. The canines and premolars are missing. The space occupied by these teeth in the usual mammalian denture appears as a diastema (Figs. 1 and II). The incisors are formed and maintained by persistent enamel organs and pulps. Owing to the situation of the mandible and to varying curvature, the mandibular incisors normally articulate posterior to the maxillary incisors.[5] The glenoid cavity of the temporo-mandibular articulation is longitudinal, rather than transverse. It follows because these teeth are constantly growing, that occlusal surfaces are constantly being worn off— * * * * * *. The elongated temporo-mandibular articulation is important, since it allows the antero-posterior relation in gnawing. The proper regulation of length in the incisors (2.5 mm. average per week) is controlled by their own interaction, a very important factor in the animal economy.[7]

All molars of all rodents except the rat grow persistently from constantly functional enamel organs and pulps, and therefore must be continually worn and renewed. They are of the lophodont or hypsodont coronal pattern common to herbivorous animals and are very unlike human teeth. In the rat the molars are completed teeth of the bunodont coronal type common to the omnivora, including man.[5] They are all erupted by the thirty-fifth day[8] and are completed apically and attached by fixed alveoli by about the sixtieth day. These twelve teeth are, therefore, comparable numerically and anatomically to the molars of man (Figs. I and II).

According to the period in which defects arise, it should be possible to state approximately whether they are developmental or structural disturbances observed in adult teeth.

The association of two such divergent dental types in the same animal is exceptional in the mammalia. It has a high experimental value, for the effect of nutritional errors may be observed in the persistently growing incisors and compared with that in the fixed molars (Figs. 1 and II).

Of the numerous diets employed for the study of the relation of certain nutritive principles to bone pathology, fifty-seven were selected as affording data which would be especially worth while for the study of the relation of the diet to the development of oral structures. These were so constituted that the effect of small variations in quality of several dietary factors on the maxillæ, teeth and attachments, could be interpreted.

We became interested in the study of the relation of the character of the diet to tooth development and tooth preservation through our observations on the relation of the diet to rickets and related conditions in the rat. It was shown in the latter studies that the skeleton of the rat is extremely sensitive to the composition of the food, especially as respects its content of calcium, phosphate, and fat-soluble A, and to another organic factor which is contained abundantly in cod-liver oil, and less abundantly in leaves of plants, but which occurs only in insignificant amounts in the cereal grains, legume seeds, muscle meats and most other common foods in use in the temperate regions. Faulty relations between these factors induce remarkable deviations from the normal histological structure of the bones, and our studies have shown that the young rat is extraordinarily sensitive to defects in these factors in the diet.

Casual observations had long ago shown us that our experimental animals which fell short of the optimum in their physical development had teeth which were poor as compared with those of animals on diets of excellent quality. It was, therefore, decided to study in a systematic way the teeth of a series of groups of rats which were prepared primarily for the purpose of securing bones for histological examination. This study of rickets and related conditions was a very comprehensive one, embracing a series of diets which were modified in more than seven hundred and fifty different ways. Up to the present time we have prepared and examined about seventeen hundred animals, representing a wide variety of diets, the biological values of every factor of which we have been able to evaluate with a fair degree of accuracy. The detailed study of this series of skulls requires considerable time and will be reported later. In the present paper we present a preliminary study of 220 animals, which were typical of groups restricted to fifty-seven diets variously modified.

It is now possible to study the dietary factors in their relation to tooth development and to permanency of these structures under circumstances far more favorable than hitherto, because we now are in possession of much knowledge concerning the nutritive needs of an animal, and also of the dietary properties of a large number of our more important foodstuffs. Furthermore, the specific

effects of faulty diets of many kinds have been carefully observed, and in the case of rickets we have an extensive knowledge of the chemical causes underlying a pathological syndrome of great significance. In this and future communications we shall present evidence which can be interpreted in no other way than that the developmental factor is an exceedingly important one in determining the quality of the dental structures, and that the composition of the diet is of profound significance both in forming and maintaining good teeth and supporting and nourishing attaching tissues.

For more than twelve years we have maintained our breeding stock of rats on a monotonous dietary regimen, which experience had taught us was more satisfactory in inducing vigorous growth, high fertility, and success in the rearing of vigorous young, than could be attained by feeding table scraps, dog biscuit, bread and milk, or a mixture of grains, forage plants, and of foods of animal origin, where a freedom of selection was permitted the animals. With this diet we have been so successful that we have steadily improved our animals from year to year, not alone through uninterrupted nearly optimal nutrition, but also through rigid selection, year by year, of the most perfect specimens for renewing the breeding stock. We have employed animals from this stock as a standard of excellence with respect to dental structures. The experimental animals were of the same stock (their own progeny), but reared on diets which deviated in some one or more factors from the ideal relationship or composition essential for optimal nutrition. One criterion of the degree of success which we have attained in the feeding of our breeding stock is the nearly complete absence of dental lesions in most of the animals from these controls, even though they were twelve to fifteen months old, or nearly half way through the extreme span of life of this species.

The stock ration consists of a mixture of wheat, maize and rolled oats, 30 per cent each, flaxseed oil meal 10 per cent, whole milk *ad libitum,* fresh cabbage or carrots twice a week. The seed mixture was ground together so finely as to preclude the possibility of the animals picking out and eating the components separately.

The experimental diets employed in these studies are tabulated and described in Tables I and II. For detailed discussions of these diets reference should be made to the charts.

Nutrition studies in recent years have brought to light the fundamental principles upon which quality in foods is to be interpreted. Present day studies are revealing how remarkably sensitive is the protoplasm to deviations from the optimal composition of the diet with respect to several factors. No one suspected until very recently that adherence even for a brief period to an improperly constituted diet could induce changes in the histological structure of any of the body tissues which would be possible of demonstration by histological technic. Our studies of the effect on the osseous system of changing the concentration and relative proportions between calcium, phosphorus, and one of the vitamins (fat-soluble A) in the food supply have shown that aberrant growth of the several anatomical elements in the bone can be easily induced. This is accomplished without making the deficiencies or excessive concentration of one or another of these nutritive principles greater than is likely to occur in the nutrition of American families at the present time.

It has been abundantly proven that the essential components of an adequate diet for man are the following:—Protein of good quality in sufficient amount. This is equivalent to saying that the protein of the diet must furnish sufficient amounts of each of the eighteen or twenty amino-acids essential for the construction of the tissue proteins. It must contain a source of the sugar glucose. This may be starch, cane sugar, malt sugar, milk sugar or dextrins. It must contain nine mineral elements in appropriate combinations. These are sodium, potassium, calcium, magnesium, chlorine, iodine, phosphorus, sulphur, and iron. These may all be furnished in the form of common mineral salts except sulphur, which must be furnished to the body in the form of the amino-acid cystin, which is contained in most proteins. In addition to the above named substances, the diet must furnish three, and probably four, substances of unknown composition which we designate collectively as vitamins, and specifically as fat-soluble A, water-soluble B, and water-soluble C, and a calcium-depositing factor. There is no experimental evidence in support of the view that man or animal actually requires fat in the food. At present it is not possible to introduce fat-soluble A into the diet without also introducing some fat, so this matter is unsettled.

It will be seen, therefore, that the diet may be remarkably simple in its construction and still meet all the needs of the body. We now appreciate, however, that even a wide variety of foods from certain sources yield diets which are not properly constituted for the maintenance of satisfactory nutrition, either in the growing young or the adult. This appreciation of the damage which may result from taking a diet in which the content and proportions among certain inorganic elements, notably calcium and phosphorus, and the content of one or another of the vitamins, differentiates sharply the present day viewpoint of students of nutrition from that of workers in this field a decade or more ago.

It is especially significant from the standpoint of prevention of oral disease that our knowledge of the pathological conditions resulting from faulty diets is more complete in respect to the development of the osseous system than in respect to any other syndrome. It has been shown experimentally that diets resembling those commonly used in American homes produce profound

alterations in skeletal development. This occurs whether the diet be fed directly to the young as soon as they can be safely weaned, or to the mother during pregnancy or the nursing period. The prevalence of rickets in babies and young children suffices to convince even the most skeptical that we are in ordinary practice actually feeding our infants and children so as to induce malnutrition of this type in very numerous cases. Since the teeth are in a way closely related to the osseous system, it is to be expected that faulty diet should play an important rôle in causing poor development of the dental apparatus.

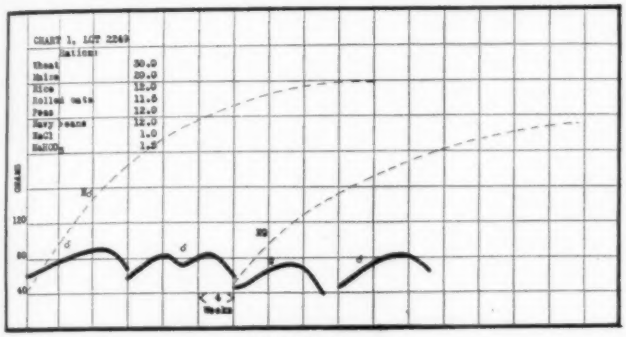


CHART 1.

Lot 2249 was fed from weaning time on a diet of cereal grains and legume seeds (peas, beans), supplemented with sodium chloride and sodium bicarbonate. The latter salt was added because the cereals all contain an excess of acid-forming elements (chlorine, phosphorus, sulphur) over basic elements (calcium, magnesium, sodium, potassium), and the amount added was sufficient to make the diet as a whole slightly alkaline.

The chief fault in this diet was lack of calcium. According to our studies, this mixture of cereal grains and legume seeds contained only about one-fifteenth of the optimal calcium to meet the needs of the growing rat. It contained only about half the requirements of fat-soluble A for this species. The protein content was somewhat below the optimum, both in amount and quality, and the diet could be enhanced by the addition of phosphorus, although the deficiency in this element was not marked.

On this diet the animals grew very little and declined and died after about three months. They were short, stocky, and rough coated. Their bones were poorly developed.

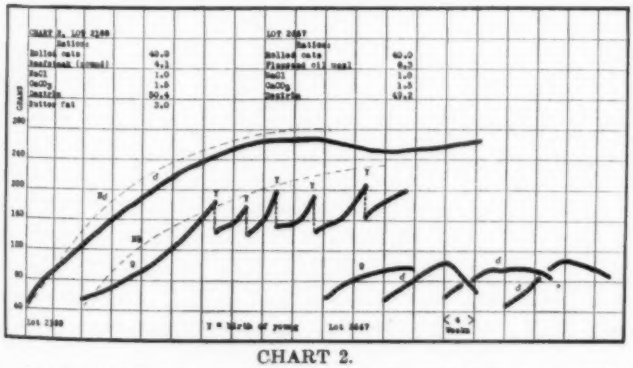


CHART 2.

Lot 2188 had from weaning time a diet containing but 9 per cent of protein of fairly good quality. There were no inorganic deficiencies, since the beefsteak contained phosphorus in liberal amount, and calcium carbonate was added in quantity sufficient to make the calcium content near the optimum. The 3 per cent of butter fat served as a source of fat-soluble A and furnished enough of this factor to support good growth.

The animals grew fairly well, but never reached the maximum size. Their fertility was high, but practically all the young died. Their coats became rough and they looked old at an early age. The cause of this lay in insufficiency of the protein content of the diet.

Lot 2667. The diet of these animals contained 9 per cent of protein of rather poor quality. It was very low in fat-soluble A and in phosphorus, but contained about the optimal amount of calcium.

On this diet opthalmia developed in from five to eight weeks because of the lack of fat-soluble A. The animals grew but little, and died after about three months. Their coats were never sleek and they presented a poorly nourished appearance. The bones of these rats were poorly nourished.

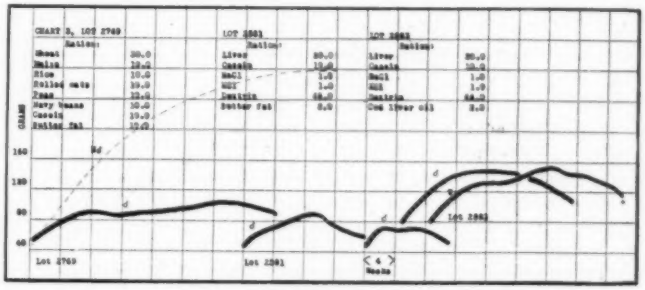


CHART 3.

Lots 2769, 2581, and 2883 may be considered together, since the only deficiency in any of them was lack of calcium. Casein enhanced the quality of the vegetable proteins in Lot 2769. In Lots 2581 and 2883 the casein and liver sufficed as an excellent source of this factor. Butter fat and cod-liver oil furnished fat-soluble A. All other factors except calcium were satisfactorily constituted. When suitable amounts of calcium carbonate are added to any one of these diets, good growth and high fertility are secured. Without calcium additions but little growth was possible, and no young were secured in any of the groups in each experiment. The curves in these charts are those representing the growth of a typical individual in a group.

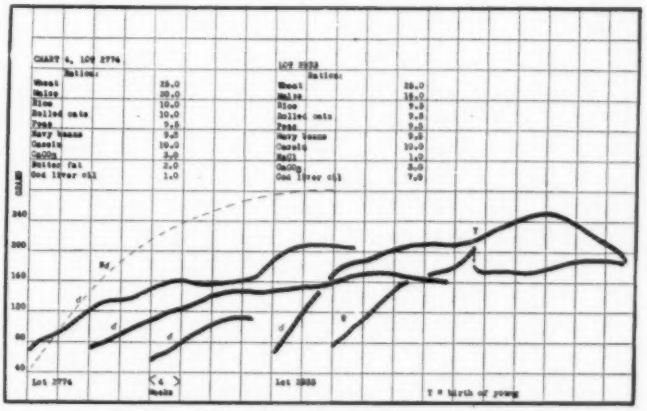


CHART 4.

Lots 2774 and 2933. These two groups had diets which were well constituted except that each contained about double the optimal amount of calcium. For this reason their growth was somewhat depressed and their fertility was interfered with. They had poor coats and did not look well nourished.

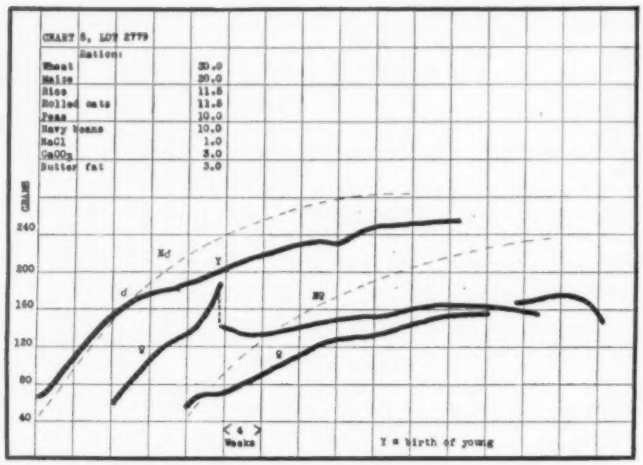


CHART 5.

Lot 2779 had a diet containing double the optimal amount of calcium. The phosphorus content was too low. It should be remembered, however, that a high content of calcium in the diet is tolerated better if the phosphorus content is also high. The content of fat-soluble A is sufficient to enable the animals to grow well, provided all other factors in the diet are properly adjusted, but more of it would have aided the animals in this experiment to tolerate better the excessive intake of calcium and the faulty proportion between this element and the too low phosphorus supply.

These animals aged early, had poor coats and appeared poorly nourished. The fertility was low and all the young born died early. They should be compared with Lot 2775 (Chart 6), where the diet is similar, except that the latter received 10 per cent of a purified protein, casein.

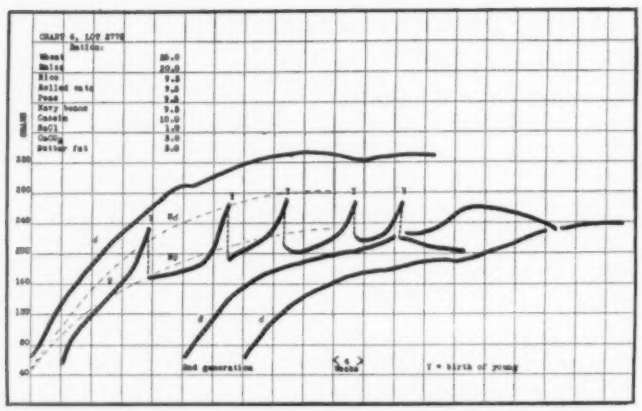


CHART 6.

Lot 2775 had a diet which was well constituted except that the calcium content was too high (approximately double the optimum). They grew well and their fertility was high, but the infant mortality was also high. Several young which were reared were continued on the diet. Their growth was somewhat stunted. An excessive intake of calcium causes early deterioration. It should be appreciated, however, that the optimal calcium intake for the rat is approximately fifteen times the content of this element contained in the cereal grains.

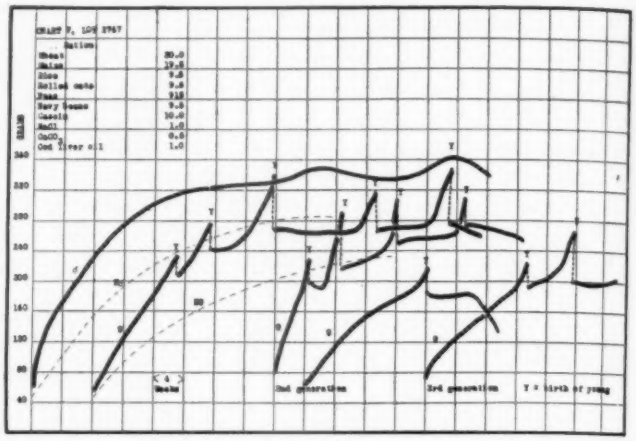


CHART 7.

Lot 2767. This diet was well constituted in every respect except that the calcium content was a little less than half the optimum. The cod-liver oil supplied a high content of fat-soluble A, and this aided greatly in enabling the animals to tolerate the deficiency of calcium in their diet. We believe, however, from experience with other experimental diets, that double this amount (2 per cent) of cod-liver oil would have further contributed to their physiological well-being.

Growth was excellent in this group. The fertility was high and the infant mortality was low. These are the criteria of good nutrition. The second or third generations of animals reared on this diet showed no definite signs of inferiority.

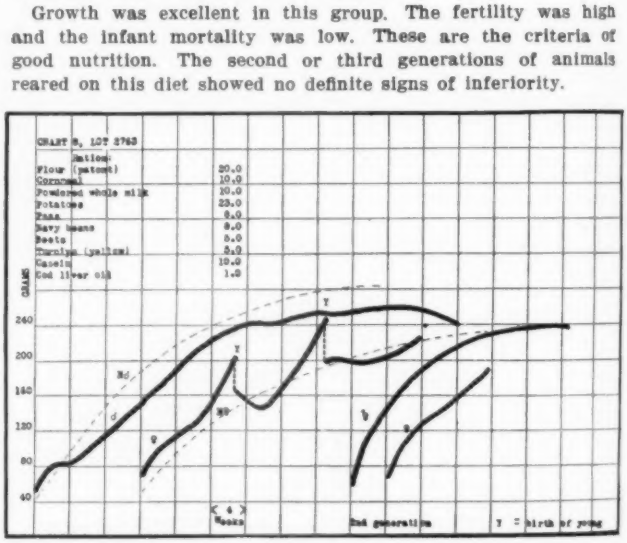


CHART 8.

Lot 2763. The diet of this group presents some very interesting features. It consisted in large part of milled cereal products, tubers and fleshy roots (potatoes), whole milk powder (Merrell-Soule), a purified protein, casein, and cod-liver oil. The last substance was included for the purpose of adding one of the vitamins (fat-soluble A). This diet was very comparable to the better class human dietaries composed of wheat flour, potatoes, meat, and butter. The meat in this diet was replaced by casein. Both are essentially sources of protein and phosphorus.

Growth was only fairly successful, the fertility was rather low, and the infant mortality somewhat excessive. The second generation animals were not so vigorous as their parents.

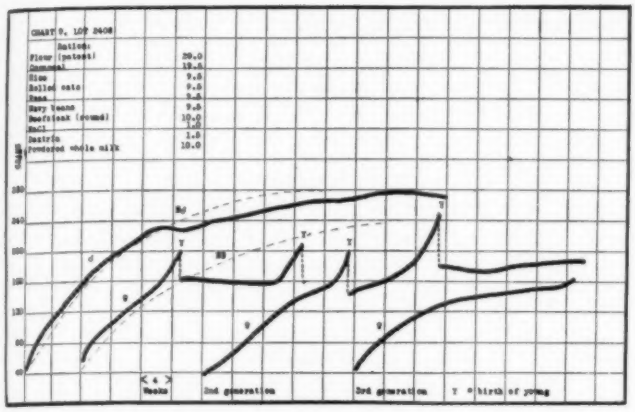


CHART 9.

Lot 2408. This diet was very comparable to such as are usually regarded as of good quality for human beings. It consisted of milled cereals, legume seeds, milk and meat. This type of diet has been extensively studied in our laboratory. It does not contain sufficient calcium or the vitamin fat-soluble A. These faults are sufficient to cause rats confined to the ration to fail to reach the maximum size or to exhibit maximum fertility. When a family is confined to such food supply through several generations, the physical development gradually deteriorates. They show signs of senility rather early and fail to maintain a well-nourished appearance. It is only necessary to introduce more calcium and more fat-soluble A into such a diet in order to change entirely the life history of the individual and of the family restricted to it.

Such diets as are described in connection with Charts 8 and 9, and which correspond rather closely with diets common in American homes, form a very important problem from the standpoint of health and prevention of oral disease. Menus corresponding to such food mixtures look attractive and appear to satisfy the appetite. An example will illustrate this.

BROILED STEAK MASHED POTATOES
BUTTERED PEAS COMBINATION SALAD
(gelatin, peas, carrots, etc.)
PICKLED BEETS
BREAD BUTTER COFFEE
APPLE PIE CHEESE

Such a diet is unsuited for the developing child or for the maintenance of optimal well being in the adult over a long period. Under such a dietary regimen early aging and inefficiency are to be expected.

In order to correct the faults in diets of this type, liberal amounts of dairy products should be included in their various acceptable forms, as butter, cheese, milk, creamed soups, and vegetables of the leafy type in the form of greens and salads. Most important among these are spinach, turnip and beet tops, celery leaves, romaine, collards, lettuce, cabbage, endive, etc. Since our ordinary diets are practically lacking in the antiscorbutic substance (water-soluble C) it is very desirable that some raw fresh fruits or raw vegetables enter into each day's dietary. These may best be used in the form of salads, since this affords an opportunity to introduce further leafy vegetables into the menu in small amounts.

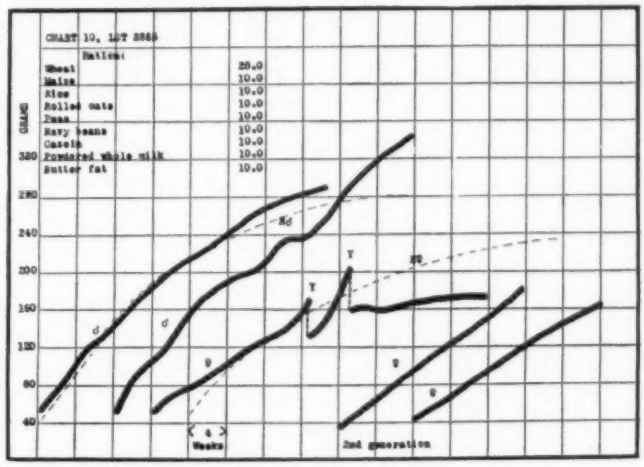


CHART 10.

Lot 2865 had a diet similar to that of Lot 2408, but whole cereals were employed instead of milled cereal products. The chief difference lay in the high content of fat-soluble A (in butter fat) in ration 2865. This improvement was of considerable significance, however. The growth was faster and the animals were in better condition than were those of Lot 2408, which had less fat-soluble A. The diet of Lot 2865 was, however, too poor in calcium, notwithstanding its content of 10 per cent of milk powder. This deficiency was serious enough to make the growth somewhat slower than the maximum rate, and the second generation failed to grow normally.

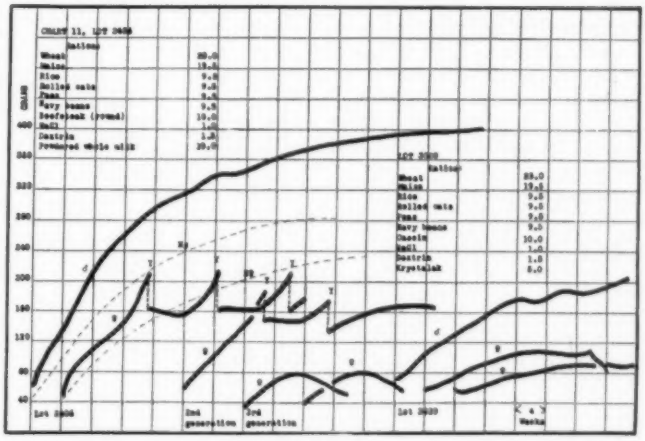


CHART 11.

Lots 2406 and 3020 represent records of two groups of rats on diets of some complexity, but in which the faults were of a twofold nature. These were insufficient calcium and fat-soluble A. The chief source of both these dietary factors in both diets was the milk powder. In Lot 2406, 10 per cent of whole milk powder, which contains the butter fat, was included. In lot 3020 but 5 per cent of skim-milk powder was included. In other words, ration 2406 was better constituted with respect to both these factors than was ration 3020. The effect of these differences in the two diets is easily seen in the character of the growth curve and the records of reproduction.

Although Lot 2406 grew well and were fairly fertile, the infant mortality was high and they aged early. Such young as grew up were maintained on the family diet but were inferior

to their parents. They were short and stocky, and rough coated, and their span of life was short.

Lot 3020, the diet of which was enhanced with respect to calcium by 5 per cent of skim milk powder, and had no supplementary source of fat-soluble A, was inferior. They were stunted in growth, and infertile, rough coated, and aged early.

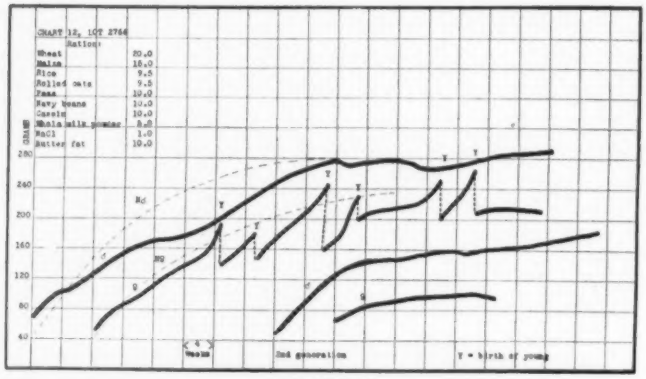


CHART 12.

Lot 2766 had a diet in which there was but one fault of any magnitude, viz., lack of sufficient calcium. It cannot be too strongly emphasized that cereals, legume seeds, tubers, fleshy roots, and meats are inadequate sources of calcium. Five per cent of whole milk in the diet did not furnish enough of this element.

The fairly good showing of these animals with respect to growth and fertility was in no small measure due to the high butter fat content of the diet. Our studies of rickets have made it clear that when the content of calcium in the diet is below the optimal, or there is a serious disproportion between the calcium and the phosphorus content in the food, the presence of a high concentration of fat-soluble A tends in some measure to protect the animals against the effects of the inorganic defect.

These animals grew somewhat slower than the optimal rate. Their fertility was fairly high and the infant mortality was excessive. The few young which were weaned never appeared sleek and well nourished. Their forms were too stocky and their growth stunted.

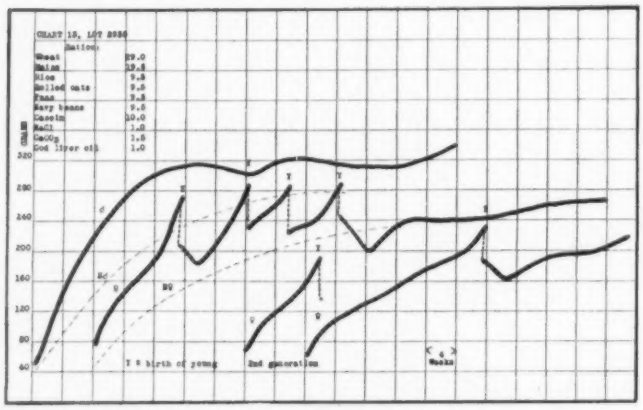


CHART 13.

Lot 2838 illustrates the effect of supplementing diets of the type discussed under Chart 11, Lot 2406, with the optimal addition of calcium in the form of carbonate. Fat-soluble A was furnished by 1 per cent of cod-liver oil. The contrast in the life histories of the two groups of animals under discussion illustrates clearly the importance of having all factors in the diet properly constituted. In other studies from our laboratory similar contrasts are brought out as the result of making success or failure of groups of animals turn on the quality of factors other than those discussed in this paper. It may be confidently asserted, therefore, that if the diet deviates even in a slight degree from the optimal in respect to any essential factor, the results will become apparent in some degree at some time in the life history of the individual.

Technique. Specimens were prepared by boiling or by maceration (rotting). Of the 220 rats included in these observations, about two-thirds were put on experimental diets at the age of forty to fifty-five days, and were continued on these diets until killed. Many adults were about one hundred to one hundred and fifty days old. No group of less than two rats of the same group was considered, and many groups contained seven upon the same diet. All rats were closely related, since our colony has been inbred without exception over a period of twelve years.

Laboratory observations and the condition of the teeth indicated that the rats had thoroughly masticated the food.

Only gross defects, under the following broad headings, are reported.

Molars *I*—Caries-like lesions.
" *II*—Loss of attaching tissues and teeth, or both.
Incisors and Molars *III*—Fracture.
Incisors *IV*—Osteodentin and pulp exposure.
" *V*—Overgrowth.
VI—Loss of maxillary and mandibular areas.

Percentages of defects were calculated from the number of rats and on the diets and number of teeth involved. Caries-like lesions occurred only in the twelve molars, while fractures occurred in molars and four incisors or sixteen teeth in all.

Under the heading "Caries-like defects in molar group," in Table III, is shown the incidence of these defects in rats restricted to the several types of defective diets discussed in the paper.

Attention is called to the remarkably constant percentage of defects throughout the series, while no caries-like defects were observed in stock rats in many generations.

These lesions, which always proceed from without inward, do not result from hypoplastic enamel. They are not absorptions by dental pulp function but have every macroscopic characteristic of caries (Figs. III to VII). That the defects which occurred in the teeth of these experimental rats were comparable to those found in human dental caries is seen in the minute and slowly enlarging initial lesion in any deep enamel sulcus: the rapid dentinal invasion undermining enamel walls; the typic laminated carious dentinal detritus and the final pulp exposure, followed by a subapical bone involvement and alveolar abscesses (Figs. VI and VII).

We believe that further microscopic study, now under way, will confirm these observations. Dentinal resistance preventing pulp exposure appears to be of the highest

order. Fractures frequently resulted from caries-like lesions, but caries rarely invaded previously fractured surfaces or roots.

The incisors even when hypoplastic or dystrophic are not involved by caries-like defects. Possibly this may be due to (1) the simple tooth form and constant use in gnawing, which prevents food debris retention, (2) the excellent resistance maintained by persistent enamel organs and pulps. If such invasion did occur, complete repair might be expected from the same source.

The majority of lesions arise in distal sulci of the first and central sulci of the second molars. These sulci are much deeper than those in the third molars and caries-like defects may result from food retention (Figs. II, V, VI, VII). Occasionally mesial and distal surfaces are secondarily involved, but, initially, interproximate contact facets are not attacked as in the human (Figs. III and IV). The excellence of contact maintained by attaching tissues may explain this immunity.

The rat's life span of almost three years is said to be comparable to man at the age of ninety. One day, therefore, approximates thirty in the human. No doubt developmental cycles vary greatly, but rats from 120-150 days old may be compared with children between the tenth and thirteenth years.

The teeth of 85 per cent of all school children between these ages are said to be 25 per cent carious. The teeth of 31 per cent of this group of rats were 5.26 per cent carious; an average of twenty-four teeth in children as compared to twelve teeth in rats were exposed to caries-producing agencies. Hence, the disparity between 5.26 per cent for the rat and 12.5 per cent for the child is less than it appears.

The periods during which rats are fed on defective diets, and during which teeth are subject to damage, are important. These vary greatly with different diets and groups, and depend upon the survival of the rat. An example is seen in the extremely rachitic and ophthalmic groups, where they rarely survived over forty days, whereas on the stock diet the animals were vigorous for seventeen months or more.

In the entire group surveyed, the longest period was 510 days for the stock and the shortest 25 days, the average being 260 days. The first and second molars, those generally involved, are fully erupted about the 25th day.

All experimental rats were placed upon these diets at an average age of fifty days. It is a rapid carious lesion indeed which involves a tooth in twenty-five days.

There is no reason to conclude that caries is any more rapid in the rat than in the human. On the contrary, from the resistance inherent in the rodent denture, we believe it to be less rapid. The whole process is relative and can be determined only by study of the individual percentages and diets which are available.

The supposed relation of sugar to human dental caries has been postulated. It is interesting to note that cane sugar does not exist in the fifty-seven diets fed to these groups of rats (See tables I and II).

Certain diets contained 5 or 10 per cent of powdered milk and 5 per cent of beets, but the small amount of milk and beet sugar contained in these is negligible. If these lesions, after further microscopic study, show the well known histopathology of human dental caries (Figs. VI and VII), it might be concluded that cane sugar was not an essential factor in these carious processes.

The diets all contained an abundance of starches, which might perhaps, be regarded as factors.

Under the heading "Loss of attaching tissues and teeth, or both, in molar group," in Table III, is shown the incidence of these defects in rats restricted to the several types of defective diets discussed in this paper.

Attention has been directed to the remarkable stability of the hard and soft attaching tissues of the molars in the rat. The flare of roots to meet occlusal stress is great, if we may judge by occlusal facets. The stout alveolus and well adapted gingival crests all indicate that such teeth cannot be lost without great damage to attachments (Figs. I and II).

It cannot be said that lack of use causes attaching tissue defects. Whether the diet be soft or hard, the molar cusps of a normal rat one year old are abraded like those of a man of fifty years.

Only such gross osseous defects as actually exposed cervical aspects of roots, or finally caused loss of teeth, are reported, and it is assumed that soft gingival tissues had been involved for some time. A microscopic study of these gingival crest lesions should be interesting and will be reported later.

Osseous defects fall into two groups: (1) the atrophic type with progressive, horizontal and regular loss of the alveolar crests, the adjacent cortex being hard, rounded and smooth (Fig. VIII); and (2) the infective type, with irregular, vertical invasion of the alveoli, and localized root exposure, in which the cortex is rough, soft or missing as in true exudative gingival lesions (Fig. IX). In fact, all phases of gingival disease occur as in the human (Fig. X).

Age and the dietary periods must be considered in individual groups in classifying attaching tissue lesions. In man gingival disease generally involves the adult, and thirty-two teeth must be compared with twelve molars in the rat. There are, therefore, 37.5 per cent less teeth exposed to the agencies which induce gingivitis in the rat than in man. The percentage of human gingival disease is not known, but a forty-one per cent involvement of the attachments of the teeth of these experimental animals, many of which were quite young, is significant.

In Table III, under the heading "Fractures in Incisors and Molars" is shown the incidence of this type of injury in teeth, resulting from several types of dietary faults.

A small percentage of fracture (0.024) will be noted in stock rats. This is not unusual in the molars of foraging rodents. Bebb's exhibit (Northwestern University Dental Museum) illustrates these conditions in wild rats. The damage, however, is inconsiderable when compared with the percentages here reported in the group on experimental diets. Such a high incidence of fractures in wild rats would seriously interfere with the nutrition.

Although growing persistently, there are no evidences of repair if incisors are badly fractured. Frequently these teeth are so brittle as to fly to pieces when seized with dissecting forceps or so chalky as to crumble if touched (Fig XI). Fracture of the incisors is commonly described as existing in rodents where the endocrine glands have been removed or damaged.

Many of the defects in maxillæ and teeth ascribed to disturbance of endocrine function have been produced in these experimental rats solely by defective diets. In animal experiments all dietary factors must be perfectly evaluated before conclusions of any value as to the relation of oral disease to nutrition can be obtained.

One of the conclusions reached by G. V. Black as the result of physical tests of human teeth, was that resilience and resistance to stress rested in the organic matrix and not in the inorganic content of the dentin. That any substance so resilient as the orthodentin of the adult rat should be so modified by diet as to become chalky or brittle is remarkable. This is conceivable in the incisor group where the vascularity of the persistent pulp might modify the dentinal matrix through the tubuli and fibrils; but the causes are not so apparent in the stable pulps and fixed dentin of molars in which the greatest percentage of fracture occurs (Figs. XI, XII and XIII).

Increasing brittleness of human teeth has been frequently observed as senescence advanced. Extremely hard and brittle teeth are common in all life periods of many races, notably among the Italians (Sicilians).

A histologic study of the matrix of such dental types as compared with brittle rat molars would be valuable if the actual diets of the people were known.

In Table III, under the heading "Pulp Exposure and Osteodentin in incisors," is shown the incidence in teeth of these abnormalities in relation to faulty diets.

Pulp exposure in molars is not mentioned, for it is negligible except that which results from caries-like lesions (Figs. V, VI and VII). Exposures which were produced by fracture have already been discussed (Fig. XI). These were quite open, easily detected, and differed materially from exposures here classified.

In normal incisors of the adult rat at least four millimeters of sound orthodentin exists between the pulp apex and grinding surface (Fig. II). If this is not normally renewed, or if it is replaced by osteo-dentin, such defects are significant of failure in function of the persistent pulp (Fig. XIV). In these conditions the pulp chamber is enlarged in all directions and dentinal walls are thin with consequent shortening of the curvature of roots. The enamel is rarely hypoplastic, though it often becomes mottled or loses its color (Fig. XV). This is evidently a dentinal condition comparable to osseous disturbance, associated with enlarged medullary spaces thin cortices and the formation of osteoid.

In Table III, under the heading of "Over-growth in Incisors" is shown the incidence in teeth of this abnormality in relation to several types of faulty diets. Overgrowth is said to result from deviation or protrusion of jaws, or loss of opposing teeth. It is also caused by pulp exposure and disease of molar attachments, both of which produce painful occlusion, and interfere with normal wear of cutting edges, which must equal incisor growth which averages 2.5 millimeters per week.[7, 8]

Addison and Appleton [7] report a series of studies on rats, confined in glass jars, presumably to prevent gnawing. The incisors were found to be as much worn by their own interaction as were those in rats eating hard food.

They note the fact that when rats were fed on bread and milk, examples of over-growth in incisors were frequent, but when placed on varied diets, over-growth rarely occurs.

An elongation of the incisors sufficient to prevent contact of grinding surfaces in the molars, which do not grow persistently, may induce malnutrition, if not starvation, in the rat. In the stock rats the elongation was not over 2 millimeters, while in the experimental rats the maxillary incisors were frequently so overgrown that the recurved cutting edges perforated the skull (Fig. XVI).

In Table III, under the heading of "Maxillary and Mandibular Damage" is shown the incidence in jaws of lesions of these types and their relation to several types of faulty diets.

The histopathology of these defects will probably be found to vary from osteoporotic and osteosclerotic states through the phases of bone pathology, similar to those we have described in studies of rickets and related conditions.

The cortex of the mandible and maxilla was thick, roughened, distorted or sclerotic with much repair, or it was canaliculated and porous or had completely disappeared (Figs. X to XVII). The medulla was much enlarged and frequently the entire bone was spongy and light with many trabeculæ. The affected areas were remarkably symmetrical and bilateral.

In the mandible, the cortex of each ramus was frequently missing along the incisor root insertions, and root apices were exposed on the external oblique lines (Fig. XVIII).

In extreme cases resorption of the coronoid, condyloid and angular processes occurred, and the rami were fre-

quently so softened that it was questionable whether the mandible could have functioned in mastication (Fig. XVIII).

In the maxilla there was loss of the premaxillary buccal plate, exposing incisor root insertions. Median separation of the premaxillæ and palatal processes occurred, and absorption of the palate bones in the floor of the nares was not uncommon (Fig. XIX).

In both mandible and maxilla, bilateral areas of resorption appeared lingually and buccally in the body of the bones, which could not have been caused by dental disease (Fig. XVIII). These must be sharply differentiated from enlarged sockets and tracts of previous fistulæ in the subalveolar bone induced by gingival disease or alveolar abscess (Figs. VII, IX, XVII).

The defective maxillæ of these rats should be compared by radiographic study with the maxillæ of children suffering from lack of development of the jaws, maxillary and mandibular protrusion and malocclusion, for it is likely that these osseous defects may be correlated (Figs. XVII and XVIII).

It is next to impossible for the orthodontist to stimulate bone growth or establish normal occlusion in the jaws of these children. The teeth may be placed in normal relation by appliances but as soon as these are removed, the entire denture drifts back into malocclusion.

In adult maxillæ, rarefied areas surrounding the apices of well filled pulpless teeth are discovered by accurate radiography. These are interpreted as alveolar abscesses and oral infective foci, and the teeth removed as a possible cause of focal disease (Fig. VII).

The contents of many such areas are frequently found to be sterile by the most careful bacteriological technique.

It is possible that these rarefactions, which also are often located in parts of the maxillæ other than those adjacent to the teeth, may arise from dietary deficiencies and defects in man as they apparently result in these experimental rats (Fig. XVIII).

In Table III, under the heading "Total Damage—Averaged from the Six Groups of Defects and Fifty-seven Diets," is shown the incidence of damage in relation to specific types of faulty diets. One hundred and six males and one hundred and fourteen females were placed on these diets, and 79.2 per cent of the males and 84.1 per cent of the females were found to have oral defects.

Twenty-seven females reared litters successfully but 92.2 per cent were orally defective.

The bone ash of the hind-leg, pelvis, scapula, and mandible of groups of rats on each of the fifty-seven diets was carefully determined. In stock animals with good bones and teeth, the ash content averaged 66 per cent for hind-leg; 62 per cent for pelvis; 65 per cent for the scapula and 74 per cent for the mandible. In the rats on deficient diets, these percentages were from 5 to 15 per cent less for all bones, save the mandible, which was constantly from 6 to 8 per cent higher, presumably because of the presence of teeth.

With the exception of the group of diets in which the deficiencies were protein of poor quality and too little fat-soluble A, the fifty-seven diets reported in these experiments were used by two of us in association with P. G. Shipley and E. A. Park in the study of rickets and related osseous pathological conditions.

SUMMARY

As the oral defects herewith reported occurred in the same groups of rats, a comparative summary of the findings in all reports follows:

These reports have shown that the internal structure of the skeleton of the rat could be changed at will by varying the ration which the animals received. In other words, bone is an extremely labile tissue and is readily influenced by nutritional environment.[10]

Some of the faulty diets which were studied produced rickets often of an exaggerated type, others caused osteoporosis. Still others resulted in the development of the peculiar lesion, which has been called osteosclerosis.

A study of the effects of these diets on the skeleton would indicate that the growth of the skeleton was dependent on at least three substances; (1) an organic substance present in certain fats which is not identical with the anti-xerophthalmic fat-soluble A; (2) calcium and (3) phosphorus.[11]

If the organic factor is low in, or missing from, a diet, the structure of the osseous tissue is dependent on the ratio between the above mentioned ions in the diet and the circulating blood.

If calcium is present in amounts equal to, or exceeding, those which would be optimal for growth and function, if all other factors are satisfactory but the phosphate ion is low, rickets is produced.[12] The same disease results when the converse relation exists between the two ions.[13]

In other words, diets which contain optimal or excessive amounts of calcium but are low in phosphorus and the organic factor, produce rickets.

Rickets also results from feeding diets low in calcium and the organic factor, although phosphorus be present in satisfactory amounts.

Diets which are satisfactory except that they are deficient in the organic substance result in osteoporotic but perfectly calcified bone.

Diets which have a comparative deficiency in calcium but are very high in phosphorus and in the organic substance, produce so-called osteosclerosis, with large numbers of small imperfectly calcified trabeculæ.

Diets deficient in calcium alone produce a pseudo-rachitic condition with over-production of osteoid.[14]

The studies of diets which contain varying percentages of calcium and calcium phosphate show that the absolute amount of either ion in the diet is of relatively little importance as compared to the ratio which exists between the two. That is, normally calcified bone is produced without regard to the reduction or diminution of either calcium or phosphate in the food, provided the content of the other ion is proportionately augmented or depressed.

Conclusions

When these findings in the bones are compared with the foregoing table of average damage on all diets, we note the following facts:

The percentage of oral defects was greatest in those rats fed the diets included under the first heading (Tables I and II), i. e., those deficient in protein, calcium and fat-soluble A. The rats on diets low in calcium exhibited the next highest incidence, and those on diets low in both calcium and fat-soluble A had the third percentage of damage.

The diets low in calcium and high in fat-soluble A, those containing low or defective protein, together with low fat-soluble A, and those in which the only deficiency was fat-soluble A, form a middle group producing 11.1 per cent oral defects (Tables I and II). The oral tissues were the least damaged by the diets high in calcium and low in fat-soluble A, those high both in calcium and cod-liver oil, and those low in calcium and in cod-liver oil (Tables II and III).

No caries-like lesions, pulp exposure or osteodentin, attaching tissue or maxillary defects occurred in our stock rats. The 0.36 per cent of defects in stock rats resulted from fracture or over-growth.

The relation of the anti-neuritic and anti-scorbutic substances to oral disease cannot be discussed in this report. A deficiency of the anti-scorbutic substance from the diet of man would no doubt be a factor in the production of oral disease but the rat is able to synthesize this substance.

Polyneuritis, xerophthalmia and scurvy are outspoken expressions of deficiency disease, which, in this country, fortunately are rarely encountered.

It is our belief, however, that severe oral disease may result from diets which are only relatively defective, where the disturbance appears to be out of all proportion to the cause.

In these border-line phases, the dietary defect or deficiency is minute and can only be determined by careful scrutiny of the diet and patient, or of the animals over a considerable period.

It is not possible at this time to name any one deficiency which specifically causes dental or oral disease; it would appear that any slight variation in the American diet, which always so dangerously approaches the level of dietary deficiency, might become active at any period of lowered resistance or of physical or nervous stress.

The present paper forms a preliminary communication of some of the results thus far obtained. We have not as yet made a detailed analysis of the relative importance of each of the essential factors of the diet in the formation of the teeth, but enough has been learned to point clearly to the diet as the agency of primary importance in determining the quality of the teeth and their vitality, as shown by the effectiveness of their barriers against invasion by micro-organisms.

BIBLIOGRAPHY

1. Guerini V.: History of Dentistry—Lea and Febiger, Phila. 1912.

2. Endelman, J. and Wagner: General and Dental Pathology—Mosby Co., St. Louis 1920.

3. Burchard, H. H. and Inglis, O. E.: Dental Pathology and Therapeutics—Lea and Febiger, Phila., Pa. 1917.

4. Miller, W. D.: Microorganisms of the Human Mouth—S. S. White Co., Phila., Pa. 1890.

5. Tomes, C. S.: Dental Anatomy, Human and Comparative —Blakiston's Sons Co., Phila., Pa. 1914.

6. Flower, W. H. and Lydekker, R.: Mammals, Living and Extinct—A. and C. Black, London, Eng. 1891.

7. Addison, W. H. F. and Appleton, J. L.: The Structure and Growth of the Incisor Teeth of the Albino Rat—Journal Morphology, March, 1915.

8. Donaldson, H. H.: The Rat, Data and Reference Tables —Wistar Institute of Anat. and Biology, Phila., Pa. 1915.

9. Black, G. V.: An Investigation of the Physical Characters of the Human Teeth in Relation to their Diseases and to Practical Dental Operations together with the Physical Character of Filling Materials—Dental Cosmos—Vol. XXXVII —1895.

10. McCollum, E. V.; Simmonds, Nina; Shipley, P. G.; Park, E. A.: Studies on Experimental Rickets. I. The Production of Rachitis and Similar Diseases in the Rat by Deficient Diets—Jour. Biology Chemistry, Vol. XLV, No. 2, June—1921.

11. Shipley, P. G.; Park, E. A.; McCollum, E. V.; Simmonds, Nina: Studies on Experimental Rickets. III. A Pathological Condition Bearing Fundamental Resemblances to Rickets of the Human Being Resulting from Diets Low in Phosphorus and Fat Soluble A; The Phosphate ion in its Prevention—Johns Hopkins Bulletin Vol. XXXII, May 1921.

12. McCollum, E. V.; Simmonds, Nina; Shipley, P. G.; Park, E. A.: Studies on Experimental Rickets. VI. The Production of Rickets by Diets Low in Phosphorus and Fat Soluble A.—Journal Biological Chemistry, 1921 XLVII, 3 507.

13. Shipley, P. G.; Park, E. A.; McCollum, E. V.; Simmonds, Nina: Studies on Experimental Rickets. XIX. Is there more than one kind of Rickets?—Amer. Journal Diseases of Children Feb. 1921—Vol. XXIII—Pages 91-106.

14. Shipley, P. G.; Park, E. A.; McCollum, E. V.; Simmonds, Nina: Studies on Experimental Rickets. VII. The Relative Effectiveness of Cod Liver Oil as Contrasted with Butter Fat for Protecting the Body against Insufficient Calcium in the Presence of a Normal Phosphorus Supply—American Journal of Hygiene, 1921, I, 4, 512.

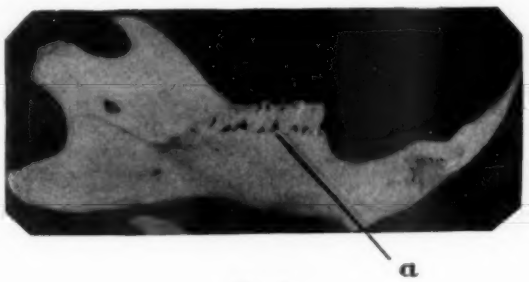


FIG. I.

FIG. I.—The right ramus of a normal mandible of a stock rat. Note the fine type of bone; the excellence and close approximation of the crests of attaching tissues (a), and the bold processes and angles. Enlarged x 3.

FIG. II.

FIG. II.—Vertical section of the left ramus, of the same mandible, showing the alveolar crests (a); the width of periodontal membranes (b); the flare of roots with pointed apices (c); the finished pulps of molars (d); and the insertion of the incisor with persistently functional pulp (e). Enlarged x 2½.

CARIES-LIKE DEFECTS

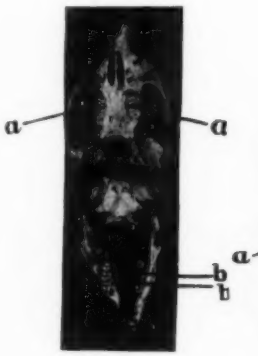


FIG. III.

FIG. IV.

FIG. V.

FIG. III.—Caries-like defects in distal sulci of maxillary molars invading interproximate contact points (a); central sulcus, caries-like defects in mandibular molars (b). Slightly reduced.

FIG. IV.—Caries-like defects, maxial and distal, first and second maxillary molars. Enlarged x 2½.

FIG. V.—Deep caries-like lesions in mandibular second molar (a) with pulp exposure. Slightly reduced.

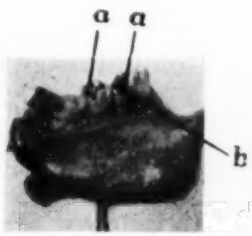


FIG. VI.

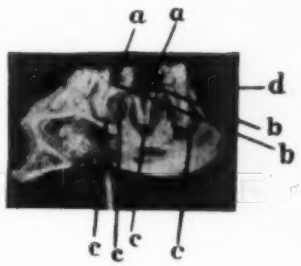


FIG. VII.

FIGS. VI. and VII.—These illustrate rami of the same mandible. Fig. VII. is a vertical section. Note the large and rapid caries-like lesions involving the right and left mandibular first and second molars (2); the pulp exposures and necrosis (b). Both first molars are dark and necrotic, with periapical abscesses and subapical bone invasion (c) via the open root canals, the alveolar crests (d) being intact. Enlarged x 3.

MOLAR ATTACHING TISSUE DEFECTS

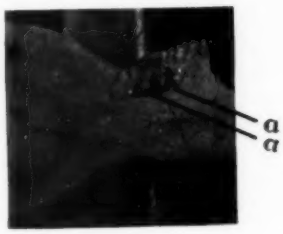


FIG. VIII.

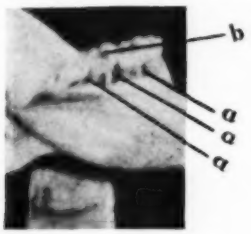


FIG. IX.

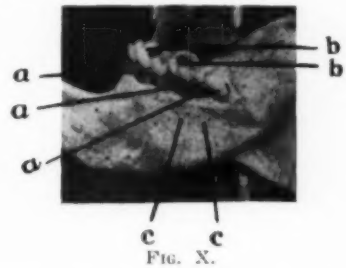


FIG. X.

FIG. VIII.—Lingual and buccal loss of alveolar crests about the second mandibular molar (2), localized and of the atrophic type—simulating gingivitis. Enlarged x 3.

FIG. IX.—Illustrating deep invasion of the lingual and interseptal crests (a); teeth tilting and caries-like lesions (b); cortex osteoporous, similating true pyorrhea. Enlarged x 3.

FIG. X.—Illustrating loss of lingual and buccal crests with deep communicating pyorrhea pockets (a); caries-like lesions (b). The alveolus is deeply and irregularly invaded, and the whole ramus is lignt, porous and canaliculated (c), simulating rickets. Enlarged x 3.

FRACTURED MOLARS AND INCISORS

Fig. XI.

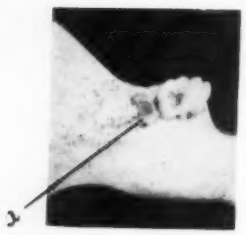


Fig. XII.

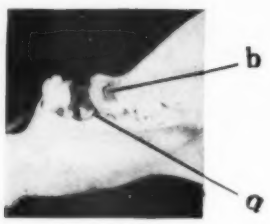


Fig. XIII.

Fig. XI.—Fractured incisors (b), and molars (a). Roots retained. No caries-like lesions. Slightly reduced.

Figs. XII. and XIII.—Rami of the same mandible: Fig. XII. shows an old fracture (a) with tilting of the much abraded adjacent teeth. Fig. XIII. shows a fracture of distal cusps in second molar (b), and first molar (a). Note that the roots are separating. Enlarged x 2½.

PULP EXPOSURE AND OSTEODENTIN, INCISORS

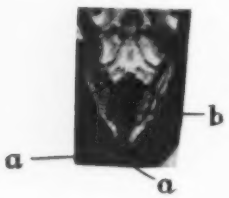


Fig. XIV.

Fig. XV.

Fig. XIV.—Osteodentin gnawing surfaces (a) of mandibular incisors; incipient caries-like defects (b). Slightly reduced.

Fig. XV.—Enlargement maxillary incisor, pulp exposure, (a); pulp chamber enlarged, enamel and apex hypoplastic, (b). General dental dystrophy.

OVERGROWTH, INCISORS

Fig. XVI.

Fig. XVI.—Complete overgrowth maxillary incisors *aa* which perforate the skull. x 3.

MAXILLARY DEFECTS

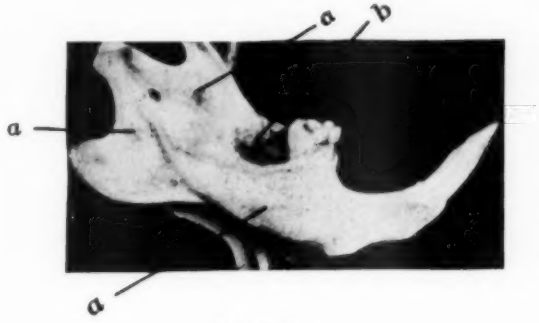


FIG. XVII.

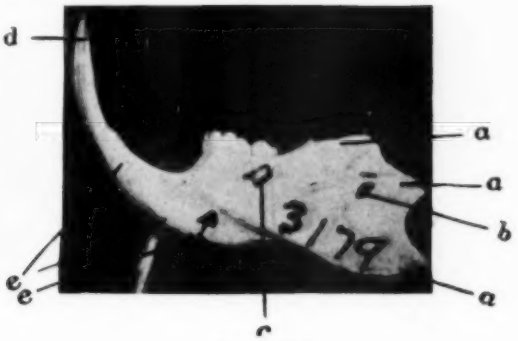


FIG. XVIII

FIG. XVII.—Left ramus, lingual view adult of rat; resorption areas (a) very thin, porous and canaliculated; old sclerotic lesion in sockets of second and third molars (b), evidently primarily infected via molar roots; compare with (c), Fig. XVIII. Enlarged x 3.

FIG. XVIII.—Left ramus, buccal view of adult rat, showing resorption of coronoid, and condyloid processes also involving the angular processes (a); exposure of incisor root apex (b); loss of cortex and incisor attachments (e); resorption of the basilar alveolar plate not resulting from dental disease (c), for teeth and attachments are normal. Compare with (b), Fig. XVII, over-growth of incisor (d). Enlarged x 3.

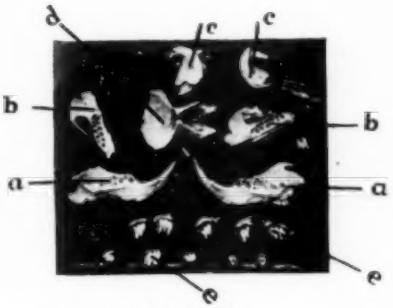


FIG. XIX.

FIG. XIX.—Disintegration of skull, maxillæ, and mandible of adult rat. Note angular absorption of the rami (a); the premaxillæ containing incisors (c); the maxillary palatal processes (b); the teeth (e), apices absorbed, all detached from loss of attaching tissues. Slightly reduced.

TABLE I

SHOWING PERCENTAGE COMPOSITION AND SOURCES OF THE EXPERIMENTAL DIETS EMPLOYED IN THESE STUDIES.

No. of ration	No. of chart	Wheat	Maize	Polished rice	Rolled oats	Peas	Navy beans	Flaxseed oil meal	Casein	Beefsteak	Liver	Milk powder	Butter fat	Cod liver oil	$CaCO_3$	NaCl	$NaHCO_3$	KCl	Dextrin	REMARKS
2249	I	30.0	20.0	12.0	11.5	12.0	12.(									1.0	1.5			Low calcium, low fat-soluble A, 12.3 per cent of poorly constituted proteins.
2188	II				40.0					4.1			3.0		1.5	1.0			50.4	Low protein (9.0 per cent) of relatively low biological value.
2667	II				40.0			8.3							1.5	1.0			49.2	Low protein (9.0 per cent) of low value Low fat-soluble A.
2769	III	30.0	10.0	10.0	10.0	10.0	10.(		10.0				10.0							Low calcium diet.
2581	III								10.0		20.0		3.0			1.0		1.0	65.0	Low calcium diet.
2883	III								10.0		20.0			2.0		1.0		1.0	66.0	Low calcium diet.
2774	IV	25.0	20.0	10.0	10.0	9.5	9.5		10.0				2.0	1.0	3.0					Twice the optimal calcium content.
2933	IV	25.0	16.0	9.5	9.5	9.5	9.5		10.0					7.0	3.0	1.0				Twice the optimal calcium content, with high cod liver oil.
2779	V	30.0	20.0	11.5	11.5	10.0	10.0						3.0		3.0	1.0				12.3 per cent of protein of relatively poor quality.
2775	VI	25.0	20.0	9.5	9.5	9.5	9.5		10.0				3.0		3.0	1.0				Twice the optimal content of calcium. Twice the optimal amount of calcium.
2767	VII	30.0	19.5	9.5	9.5	9.5	9.5		10.0					1.0	0.5	1.0				About one-third the optimal content of calcium. Slightly less than optimal content of cod liver oil.
2865	X	20.0	10.0	10.0	10.0	10.0	10.0		10.0			10.0	10.0							Low calcium diet.
2406	XI	20.0	19.5	9.5	9.5	9.5	9.5			10.0		10.0				1.0			1.5	Low calcium, low fat-soluble A.
3020	XI	25.0	19.5	9.5	9.5	9.5	9.5		10.0			5.0*				1.0			1.5	Low calcium, low fat-soluble A.
2766	XII	20.0	15.0	9.5	9.5	10.0	10.0		10.0			5.0	10.0			1.0				Low calcium diet.
2838	XIII	29.0	19.5	9.5	9.5	9.5	9.5		10.0					1.0	1.5	1.0				Optimal calcium, slightly below optimal content of fat-soluble A.

* Krystalak.

NOTE: Low calcium signifies a content of calcium derived from the natural foods included in the diet.

TABLE II

SHOWING PERCENTAGE COMPOSITION AND SOURCES OF EXPERIMENTAL DIETS DESCRIBED IN CHARTS VIII AND IX.

No. of ration	No. of chart	Bolted flour	Rolled oats	Cornmeal	Whole milk powder	Potato	Polished rice	Peas	Navy beans	Red beets	Yellow turnips	Casein	Beefsteak	Dextrin	NaCl	Cod liver oil	REMARKS
2763	VIII	20.0		10.0	10.0	23.0		8.0	8.0	5.0	5.0	10.0				1.0	Low calcium, and below the optimum in fat-soluble A. Deficient in sodium chloride.
2408	IX	20.0	9.5	19.5	10.0		9.5	9.5	9.5				10.0	1.5	1.0		Low calcium, and below the optimum in fat-soluble A. Approximately the optimal content of sodium chloride

TABLE III

TABLE SHOWING PERCENTAGE INCIDENCE OF DENTAL DEFECTS IN RATS RESTRICTED TO DIETS OF DIFFERENT CHARACTERS

No. of diets studied	Caries-like defects in molar group	Loss of attaching tissue and teeth, or both, in molar group.	Fractures in incisors and molars	Pulp exposure and osteodentin in incisors	Over-growth in incisors	Maxillary and mandibular damage	Percentage of total damage		Calcium per 100 grams ration	Phosphorus per 100 grams ration
1....STOCK RATION. No defects of a significant magnitude. Our breeding stock of rats has been confined to this diet through a period of twelve generations without any deterioration in vitality. During this interval they have improved physically because of the selection of exceptional individuals for breeding.....	0.0	0.00	0.02	0.00	1.10	0.00	0.36			
7....DIETS LOW IN CALCIUM BUT RICH IN FAT-SOLUBLE A. A diet typical of this group is described in Chart X, Lot 2865. Factors other than calcium in this diet were of nearly optimal quality.....	1.18	20.81	2.59	19.08	8.31	19.04	11.66	Lot 2865—	0.1348	0.4116
6....DIETS CONTAINING EXCESSIVE CALCIUM, AND WITH A SATISFACTORY CONTENT OF FAT-SOLUBLE A. In this group are included animals fed diets similar to or identical with those described in Chart V, Lot 2779, and Chart VI, Lot 2775. The latter differed from the former in that it contained 10 per cent of casein, and therefore furnished 0.08 grams of phosphorus per hundred grams of ration more than did diet 2779. This modification in the phosphorus content of diet 2775 improved the growth curves, and resulted in better nutrition than would the same food mixture without the added casein. This difference was not due entirely to the added phosphorus, but in part at least to the improved amino-acid supply resulting from the addition of casein. Notwithstanding these differences in the two diets, the effects on the dentition of the animals was so similar that the six diets corresponding to these types are grouped together here. We are now studying in detail the many specific problems which are suggested by these preliminary results.....	1.73	33.38	3.28	0.00	0.00	12.50	8.48	Lot 2779— Lot 2775—	1.2530 1.2482	0.3459 0.4053
5....DIETS LOW IN CALCIUM, LOW IN FAT-SOLUBLE A, AND PROTEINS OF RELATIVELY LOW BIOLOGICAL VALUE. Growth of young rats on diets of this class is very poor (Chart 1, Lot 2249). All diets of this class in which the proteins are derived solely from cereal grains and legume seeds are below the optimal in the quality of the amino-acid mixture which they yield. This means that the proteins are not so effectively transformable into body proteins as is possible with proteins of better quality	2.88	35.50	4.70	37.50	17.85	45.20	23.93	Lot 2249—	0.0216	0.3831
6....DIETS CONTAINING EXCESSIVE CALCIUM, AND CONTAINING SEVERAL PERCENTAGES OF COD LIVER OIL, OR OF COD LIVER OIL AND BUTTER FAT. Diets 2774 and 2933, Chart IV, are typical of those fed to animals classed in this dental group. The protein content was of good quality, and was abundant in amount. The calcium content was about twice the optimal. (The Optimal content is about 0.64 per cent calcium, equivalent to about 1.5 per cent of calcium carbonate added to a mixture of cereal and legume seeds). The content of fat-soluble A in these diets was very liberal in terms of the nutritive requirements of the rat.....	3.00	16.70	4.48	4.16	0.00	6.93	6.21	Lot 2774— Lot 2933—	1.2482 1.2476	0.4053 0.3983
7....DIETS LOW IN FAT-SOLUBLE A AND LOW IN PHOSPHORUS. THEY CONTAINED NINE PER CENT OF PROTEIN OF RELATIVELY LOW BIOLOGICAL VALUE. A typical example of diets of the type under consideration is Lot 2667, Chart II. They were so deficient in fat-soluble A that the animals developed xerophthalmia within a few weeks and their lives were cut short. The skulls examined were, therefore, all of animals which were chronologically young, although they showed physiological deterioration. This is reflected in the high percentage of molar defects in animals but three to five months old....	3.12	19.74	8.48	5.00	10.00	18.50	10.80	Lot 2667—	0.6668	0.2210

TABLE III---Continued

TABLE SHOWING PERCENTAGE INCIDENCE OF DENTAL DEFECTS IN RATS RESTRICTED TO DIETS OF DIFFERENT CHARACTERS

No. of diets studied	Caries like defects in molar group	Loss of attaching tissue and teeth, or both, in molar group	Fractures in incisors and molars	Pulp exposure and osteodentin in incisors	Over-growth in incisors	Maxillary and mandibular damage	Percentage of total damage		Calcium per 100 grams ration	Phosphorus per 100 grams ration
....DIETS LOW IN CALCIUM, BUT OTHERWISE WELL CONSTITUTED. The fat-soluble A content was abundant. The protein was in all cases of very good quality. The calcium content was so low as to cause in some groups stunting of growth, and early deterioration and death. (See Chart III, Lots 2769, 2581, 2883, and Chart XII, Lot 2766). In other diets small additions of milk powder raised the calcium content sufficiently to permit the animals to grow at a subnormal rate. Notwithstanding their capacity to grow, the animals on this latter class of diets (Chart X, Lot 2865, and Chart XI. Lot 2406), were physically inferior to animals on better diets. The high incidence of molar defects in these animals is an index to their nutritional instability	3.65	57.90	4.45	14.57	6.25	39.90	20.12	Lot 2769— Lot 2581— Lot 2883— Lot 2766— Lot 2865— Lot 2406—	0.0480 0.0010 0.0010 0.0904 0.1348 0.1356	0.3868 0.4000 0.4000 0.3929 0.4110 0.4301
....DIETS LOW IN PROTEIN (ABOUT 9 PER CENT) OF RELATIVELY LOW BIOLOGICAL VALUE. THEY WERE NOT FAR FROM THE OPTIMUM IN THEIR CONTENT OF FAT-SOLUBLE A. An example of the diets in this group is given in Chart II, Lot 2188. The only pronounced defects in these diets was in the protein moiety. The percentage of dental defects in rats restricted to these diets was so high that we have undertaken a comprehensive study of this factor in relation to tooth development and tooth vitality	4.74	9.47	0.08	20.82	7.72	19.71	10.91	Lot 2188—	0.6371	0.1753
....DIETS LOW IN CALCIUM, AND BELOW THE OPTIMUM IN FAT-SOLUBLE A. The calcium content of the diets in this class stood intermediate between those which derived all their content of this element from cereal grains and legume seeds, and those in which approximately the optimal content of calcium was added in the form of calcium carbonate, viz., 1.5 per cent $CaCo_3$. Typical examples of these diets are shown in Chart VII, Lot 2767, and Chart VIII, Lot 2763. All factors other than protein and fat-soluble A were well constituted	5.21	17.70	2.88	2.14	3.38	0.00	5.25	Lot 2767— Lot 2763—	0.2503 0.1817	0.4217 0.3713
....DIETS LOW IN CALCIUM, AND WITH FAT-SOLUBLE A DISTINCTLY BELOW THE OPTIMUM. The milk powder contained in these diets was not sufficient to introduce enough calcium to do more than enable the animals to grow at a rate which would seem to indicate fairly good nutrition. Actually, we know, from the results of our observations of the life histories and reproductive capacity of such animals, that they were in a state of nutritive instability. Their span of life is distinctly below the normal, and their infant mortality was high. The young were inferior in appearance and the families tended to die out within two or three generations. The percentage of dental defects was high.	5.26	14.94	1.88	37.45	11.10	13.83	14.00	Lot 2406— Lot 2408— Lot 3020—	0.1356 0.1363 0.0936	0.4301 0.3397 0.4438

TABLE IV

SHOWING THE PERCENTAGES OF ANIMALS EXAMINED, WHICH SUFFERED FROM SPECIFIED DENTAL DEFECTS.
(Data Based on Observations on 220 Rats)

Caries-like defects in molar group	Loss of attaching tissues or teeth, or both, in molar group	Fractures in incisors and molars	Pulp exposure and osteodentin in incisors	Over growth in incisors	Maxillary and mandibular damage	Total damage averaged from the six groups of defects
31.0	41.8	29.5	22.7	20.0	25.0	Males—79.2 Females—84.1

STUDIES ON EXPERIMENTAL RICKETS. XX. THE EFFECTS OF STRONTIUM ADMINISTRATION ON THE HISTOLOGICAL STRUCTURE OF THE GROWING BONES

By P. G. Shipley and E. A. Park
(From the Department of Pediatrics, The Johns Hopkins University)
and E. V. McCollum, Nina Simmonds and Ethel May Kinney
(From the Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University)

The effect of strontium salts on the animal organism has been studied by chemical and histological methods, chiefly to ascertain whether or not salts of strontium could replace lime salts in the skeleton. The therapeutic use of strontium bromide for epilepsy and kindred nervous disorders has stimulated pharmacologic studies of the toxicity of strontium salts.

The most elaborate investigation of the effect of strontium on bone development is that of Lehnerdt.[1] His conclusions may be briefly summarized as follows: (1) Strontium cannot replace calcium physiologically. (2) Strontium greatly stimulates the formation of osteoid tissue, and tends to repress the resorptive processes in the bone. (3) Strontium stimulates the osteogenetic cells.

Lehnerdt found that the feeding of moderate amounts of strontium with a liberal provision of calcium did not lead to pronounced deviation from the normal osteogenesis. If, on the other hand, strontium was added to a calcium-poor diet, the skeleton grew abnormally. The effect of the feeding of strontium was inversely proportional to the content of calcium contained in the diet.

The stimulating action of strontium on the osteogenetic tissues makes itself most evident at these points at which, under normal conditions or with a calcium-poor diet, there is greatest tendency to resorption, *viz.*, in the oldest parts of the spongiosa; at the junction of the metaphysis and the diaphysis; and on the outer surface of the medullary cavity. In these places strontium caused over-production of osteoid tissue. Lehnerdt believed that there was no abnormal formation of osteoid in the growing bone if an animal was partially deprived of calcium. He thought that only so much osteoid tissue was formed as could be calcified by the limited amount of calcium that was available. The development of new bone under these conditions was accompanied, however, by an increase in the rate of resolution of old bone substance, so that a condition of osteoporosis developed, and a fragile bone was formed, the osteoid of which was produced in so limited an amount that it could be completely calcified. He thought that calcium salts were borrowed from the old, well-calcified parts of the bones to construct new bone in the growing region, and that the thin trabeculæ were the result of forced economy in the use of calcium.

Lehnerdt believed that calcium, was, therefore, a regulator of the growth of the organic elements of the bone and that it also, impregnated the osteoid tissue in the form of insoluble salts. He considered that strontium possessed the same property as calcium, of stimulating the development of osteoid tissue, but was many times more effective in this direction. He felt that strontium, could not be used to impregnate the osteoid tissue in the form of insoluble salts in anything like the degree that calcium could, and therefore the osteoid formed under its influence remained soft. The deposition of strontium is a secondary phenomenon, which occurs as a result of a too meager supply of calcium to the bone. Strontium, Lehnerdt believed, can be absorbed only at the expense of calcium.

Lehnerdt fed his dogs strontium phosphate, adding this to a diet of muscle meat and fat. He used therefore, a diet which was not only very poor in calcium (except when calcium phosphate was added), but was very high in phosphorus. He did not appreciate the significance of this fact.

Lehnerdt found that the bones of animals fed on the above diet showed in various degrees a pathological condition which resembled rickets, in that it was characterized by over-production of osteoid tissue. It differed from rickets, however, in many important respects: (1) The line of junction of the epiphyseal cartilage with the shaft was quite regular. The cartilage neither persisted as it does in rickets, nor was it irregularly destroyed by blood vessels from the shaft. (2) The marrow cavity was nearly or quite obliterated by large numbers of trabeculæ which were made up of osteoid tissue, with little or no calcified bone in them. He felt that this lesion was brought about by an enormously increased production of osteoid tissue ("apposition") and the complete or nearly complete suppression of the resorption which goes on in the course of normal osteogenesis. (3) The gross deformity of the bones differed from that caused by rickets.

Lehnerdt was able to induce the condition in the fœtus *in utero* and also in the bones of pups nursed by a strontium-fed mother for 32 days, as well as in adult animals (dogs and rabbits). The affected bones grew in length and in diameter. The cartilaginous ends of the bones and the junction of the shaft with the head of the bone were not affected. The cartilages were calcified almost or quite completely. The line of junction of the epiphysis with the shaft was regular and sharp. The ends of the shaft were composed of a mass of trabeculæ. Each of these was surrounded by or composed entirely of osteoid tissue. Some of these osteoid trabeculæ contained rests of calcified intracellular matrix of cartilage. Others were calcium-free. The marrow cavity in the growing region was completely obliterated by the osteoid trabeculæ (sclerosis). In the shaft the marrow cavity was reduced in size by over-growth of osteoid tissue in the cortex, and the persistence of osteoid spongiosa, which should have been absorbed. Lehnerdt believed that one month after birth the bone which had formed the cortex of the bones at birth still persisted unchanged. Growth of the shaft in diameter was carried on by the development of masses of osteoid tissue beneath the periosteum.

The bones of adult and nursing animals were not greatly deformed in spite of the severity of the lesion. The ribs showed a change at the costo-chrondral junctions which differed from the rachitic change in man in that there was not much swelling of the junction or deformity of the thorax. The ends of the ribs did show a bullet-shaped swelling, of a whitish color, at the growing end of the shaft which corresponded with the region of "sclerosis." Wrist and ankle joints were not markedly enlarged.

The pathological picture of this strontium sclerosis was much exaggerated in the bones of pups of strontium-fed mothers.[2] The bones of these animals were very badly deformed and had practically no medullary cavity.

We have studied the reaction of young rats to strontium to determine the relationship of "strontium sclerosis" to rickets, and to determine, if possible, the relation of the alkaline earth ion to the production of the rachitic lesion. Our studies of the effect of strontium on grow-

ing bones were carried out in the light of a wide experience in observing the manner in which specific starvation for calcium or for phosphorus, deviation from the optimal ratio between calcium and phosphorus, and the effect of deprivation of liberal provision of an organic substance associated with certain fats, notably cod-liver oil, modified the histological structure of the bones. We are, therefore, able to interpret our results from a more comprehensive viewpoint than could Lehnerdt, who failed to appreciate the importance of an excessive amount of phosphate together with a deficient amount of calcium in the diet in modifying the structure of the bones of his animals. The significance of this will appear later.

It is essential that the character of the diet which we used in these experiments be understood, in order to appreciate the influence which we assign to strontium in modifying the structure of the growing bones. Diet 2581, which we employed as a basal one in this study consisted of

	Per cent
Liver (cooked and dried)	20.0
Casein	10.0
KCl	1.0
NaCl	1.0
Dextrin	65.0
Butter fat	3.0

one hundred grams of this diet contained .0008 gm. of calcium, and .4060 gm. of phosphorus. The weight ratio of calcium to phosphorus was 1:507.5. The atomic ratio of calcium to phosphorus was 1:654.2.

There was but one fault of a demonstrable magnitude in this food mixture (for the rat), viz., lack of calcium. Our studies have convinced us that the optimal content of calcium for the growing rat is about 0.64 per cent (Ca.). The optimal phosphorus content has not been established so accurately as has the optimal content of calcium, but provisionally we have deduced from our studies that, other factors being satisfactory, about 0.40 per cent (P) represents the minimal content on which optimal development can take place. The proteins of this diet were of excellent quality, and were abundantly supplied (24.8 per cent). Both the butter fat and the liver are rich in fat-soluble A. Liver is rich in water-soluble B. The needs of the animals for sodium, potassium and chlorine were supplied by the additions of potassium and sodium chlorides noted. When calcium was added to this diet in the form of carbonate to the extent of 1.5 per cent of the food mixture, rats were able to develop in a perfectly normal manner. This is shown by the records of Lot 2162, Chart 1. Without the addition of calcium no growth was possible on this food mixture, as is illustrated by the records of Lot 2581, Chart 2.

A study of the effects of this diet, with and without calcium additions, affords an interesting illustration of the importance of a proper balance between the calcium and phosphorus in the diet. When the formula given above was fed as the sole source of nutriment, the skeletal development was very abnormal.[3]

The diet without calcium addition failed to induce appreciable growth. The hair of the rats was rough and irregular in length. Their skulls were thin and easily compressible. The incisor teeth were brittle, yellow, and in most instances easy to extract. There was never any evidence of ophthalmia. The epiphyses of the long bones, especially at the wrist and knee, were enlarged. The shafts of the bones were slender. The thoraces were much flattened from side to side, slender, and marked by deep grooves along the costochondral junctions. The junctions of ribs and costal cartilages were enlarged, and there was marked angular deformity of the thorax. The degree of angular deformity exceeded that found in the ribs of rats fed diets low in fat-soluble A and phosphorus. The thymus was atrophied. The spleen was frequently greatly enlarged. There was little, if any, subcutaneous or visceral fat. The testes were atrophic. The long bones cut easily. There were, as a rule, no fractures to be found in any of the bones except the ribs, which were usually studded throughout their length with the calluses of healing fractures.

The bones of rats which have been on diet 2581 were in a pathological condition closely allied to rickets, but which varied in some respect from that disease as it is commonly seen in children. The bones resembled those of rachitic children in that they were characterized by the complete or nearly complete failure to form a provisional zone of calcification. They had a pathological metaphysis between the shaft and the epiphysis of the bone, and there was a great over-production of osteoid tissue with the consequent deformity of the skeleton. The epiphyseal disc of cartilage was somewhat widened, and was invaded to a slight degree by blood vessels from the diaphysis. The cartilage cells underwent degeneration and metaplasia into osteoid and the bone marrow in the growing region of the bone was entirely replaced by newly formed connective tissue. On the other hand, the condition varied from human rickets as it is ordinarily seen in several ways. The cartilage of the epiphysis was not persistent to the extent usually seen in human rickets, or in animals which have received diets which are high in calcium, low in phosphorus, and in a substance which is present abundantly in cod-liver oil. The cartilage was not as much disrupted by vascular invasion, and the columnar arrangement of the proliferative zone was not greatly disturbed. The metaphysis was narrow, and its entire arrangement was much more regular than that usually seen in human rickets of a grade sufficiently severe to induce an equivalent amount of skeletal deformity. Trabeculæ were present in extraordinary numbers in the spongiosa. The marrow cavity was encroached upon and much narrowed by the cortex, which was greatly thickened and consisted of a sponge-like formation of partly calcified trabeculæ. Signs of increased and active resorption were seen throughout the shaft, especially in the cortex and in the older part of the spongiosa. The pathology of these bones has been described at length in another communication.[3]

The condition shown by these bones was somewhat analogous to that described by Schmorl, and to which he gave the name *pseudo-rachitic osteoporosis*. It was apparently a condition which is on the borderline between the pathological picture of rickets as it is usually seen, and the lesion which is known as osteosclerosis. The latter condition may be induced by feeding certain diets containing

an abnormally large amount of phosphorus. We have shown, however, that rickets in the rat, or at least a condition which is identical with rickets from the histological point of view, may be induced by feeding diets which are low in calcium, but which contain an approximately normal amount of phosphorus.[4] A pathological picture, identical with human rickets as it ordinarily occurs, also results from the ingestion of diets which are high in calcium but low in phosphorus and fat-soluble A.[5] The disproportion between these inorganic salts in either direction must be, however, within certain very definite limits, if the bone lesion produced by them is to be identical with the rickets of children. By varying the proportion in which these ions exist in the animal's diet bones may be produced which are either normal, rachitic, or sclerotic, but it must be appreciated that the line of demarcation between the lesions is not sharply cut, and that transition states may occur between conditions of rickets and osteosclerosis, between rickets and normal bone and between normal bones and sclerosis. We have suggested in another place that the rachitic condition which is accompanied by tetany may sometimes be a type different from rickets as it is commonly seen and which tetany never complicates. We have suggested moreover, that there may be in children, as in rats, transition forms of rickets, such as rickets of the so-called healing type.[6]

In order to demonstrate the effects of strontium in the absence of calcium on the growing bones, we employed Diet 2581, the effects of which have been described above, with the following additions.

Diet 3152.

Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
$SrCO_3$	2.2
Butter fat	3.0
Dextrin	62.8

This diet contains an amount of strontium which was the atomic equivalent of 1.5 per cent of calcium carbonate in the diet, or the equivalent of 0.6 per cent of the element calcium. The dietary properties of this food mixture, aside from the strontium contained in it, have been described above.

The results of feeding this diet to young rats throw some light on the question as to the possibility of strontium being capable of replacing calcium in a physiological sense. Without the addition of either calcium or strontium young rats on this diet grow scarcely at all. With the optimal calcium addition they grow normally. Without calcium but with strontium addition equal to 2.2 per cent of the food mixture, they are able to reach twice or three times their initial weight in most cases. This is especially true when cod-liver oil is included in the diet, but the cod-liver oil did not exert a protective effect on the bones in the sense of preventing the abnormalities in histological structure. It will be recalled that cod-liver oil is extraordinarily effective in preventing the abnormal development of bones characteristic of rickets and related conditions, when these are brought about by unfavorable relations of calcium and phosphate in the food, an insufficiency of fat-soluble A, or the second organic factor associated with certain fats which our experimental data seem to demonstrate to play an important rôle in bone growth.

Animals confined to diet 3152 developed a strange condition after from 25 to 35 days, manifested by peculiarities of gait and of sitting posture. In the early stages they sat with the back rounded in an abnormal manner, and with the hind legs held in a loose and irregular fashion, as if they were suffering from a partial loss of muscular control. As the condition grew worse, the hind legs become nearly useless in walking and were dragged behind the animal. Cod-liver oil tends distinctly to protect the animals against this loss of muscular control.

Autopsy. These animals were all fairly well grown. Their coats and eyes showed nothing abnormal. The skulls were very soft. The teeth were slender, white, and fractured easily. In some animals even the molars were loose. Occasionally the incisors were broken during life. The thymus and testes were always normal. The spleen was always much enlarged. No other visceral abnormalities were found. Often there was no deformity of the thorax but sometimes the chest wall was slightly sunken along the line of the costo-chondral junctions. The shafts of the ribs were enlarged behind the junctions with the cartilages. There were sometimes healed fractures of the shafts of the ribs, but often none were to be found. The long bones showed very marked changes. Their extremities were greatly enlarged so that the wrist, knee and ankle joints appeared to be swollen. The swellings were snow-white and were so soft that free movement in all directions was permitted as though the bones were fractured on the shaftward side of the epiphysis. The extremities of the bone cut very easily, but there was a considerable resistance and grating when the shaft was cut.

On section the epiphyseal centers of ossification were small and the cartilage was much increased in depth. The junction of the cartilage with the shaft was irregular and the cartilage was invaded by large tufts of blood vessels. A broad zone of white tissue (the metaphysis) separated the cartilage proper from the shaft. The cortex of the shaft was thickened somewhat as the result of the growth of osteoid tissue. The shaft contained a relatively small number of trabeculæ, each surrounded by a zone of osteoid tissue.

Histological. On microscopic examination the most striking finding was the over-growth and persistence of the epiphyseal cartilage and the breadth of the metaphysis. The zone of resting cartilage was abnormally wide and the matrix between the cells was abnormally abundant in this region. At the beginning of the proliferative zone reproductive activity of the cartilage cells was marked, so that the abundant matrix was replaced by cells which were crowded together in a dense mass which soon lost any semblance of columnar arrangement. The proliferative zone was in many places from 20 to 30 cells in depth. At the junction of the proliferative zone with the resting cartilage the cells had a fusiform or crescent shape, and the cytoplasm and cell capsules stained intensely with basic dyes. The older cells, however, soon became globular. Unless contact was established with the blood vessels of the shaft, they retained their affinity for basic dyes. Intense activity of the

cartilage cells was apparently the first manifestation of the onset of the lesion. Calcification of the cartilage did not occur. The cartilage was invaded and destroyed in an irregular way by single capillaries and by large tufts of vessels from the shaft. Large tongues of cartilage persisted in the metaphysis. The cartilage cells degenerated or underwent metaplasia into osteoid corpusles as soon as they came into contact with the metaphyseal blood supply. Metaplasia was often preceded by active division of the cells (Figs. 2 and 3), but the daughter cells did not grow, so that the bone corpuscles derived from the cartilage were very much smaller than the parent cells.

The initial step in the processes of metaplasia or degeneration was loss of the ability of the cell capsule and cytoplasm to take up basic dyes. In the vicinity of the blood vessels many of the cartilage cells became degraded into cells of the fibroblast type Their nuclei became pyknotic and they assumed a spindle shape. (Fig. 2). In this condition they might become embedded in osteoid tissue where degeneration might go on until the entire cell disappeared and nothing remained to indicate that it had ever existed except the space occupied by it in the osteoid. Sometimes small groups of cartilage cells or single elements could be found embedded unchanged in a mass of osteoid in the metaphysis. Cartilage cells in the long tongues which persisted in the metaphysis frequently reverted to the form seen in the beginning of the proliferative zone, and became flattened and fusiform in shape. So long as these cells were protected by osteoid or other cartilage cells from the influence of the blood vessels, they retained their identity and their affinity for basic stains. At the border of the cell masses, however, the cytoplasm stained like osteoid tissue. As the blood vessels approached the cartilage cells, the cells divided rapidly within their capsules into numerous small daughter cells, and these became embedded in osteoid and indistinguishable from osteoid corpuscles (Fig. 3).

The metaphysis which formed in these bones was deeper than that in any other bones we have examined (Fig. 1). It consisted of osteoid trabeculæ and cartilage cells, unchanged, or in all stages of degeneration and metaplasia. It contained numerous blood vessels surrounded by fine connective tissue reticulum supporting a few marrow elements. Some of these vessels were very large. Finally, the masses of unchanged cartilage described above were present. The trabeculæ of the metaphyses usually contained no trace of calcium, but occasionally one was found with a bit of calcified intercellular matrix in its center.

The trabeculæ of the spongiosa were not numerous, and were usually well formed and straight. Each was more or less completely surrounded by a border of osteoid tissue (Fig. 4). The osteoid corpuscles were small. The osteoblasts surrounding the trabeculæ were in a continuous layer and were flat cells like blood vascular endothelium. The calcified cortex was slender and dense and was covered by osteoid tissue which was traversed by numerous blood vessels (Fig. 1). The medullary cavity was very little narrowed. The bone marrow cells were normal.

The junction of the metaphysis with the shaft was marked in these bones by a line of calcified intracellular substance embedded in osteoid tissue (Fig. 1,r). This line showed the situation of the epiphyseo-diaphyseal junction at the time when the first effects of feeding the strontium-containing ration manifested themselves. No signs of resorption of bone were evident.

Briefly then, the effect of feeding this ration to rats is to produce in the skeleton persistence of the cartilage, and increased formation of osteoid tissue. A pathological metaphysis is formed and the epiphyseo-diaphyseal junction is rendered very irregular. Resorption of the bones is checked during the administration of the ration. Clinically the condition is characterized by deformities of the skeleton resembling those which result from rickets in man and by partial or complete paralysis of the extremities.

We cannot discuss the cause of the paralysis at this time. We cannot say whether it is dependent on lesions of the central nervous system or on the loss of rigidity of the bones at the epiphyseo-diaphyseal junction. The changes produced in the bones are very little different from the rickets which results from feeding rats on diets in which the phosphorus content is very low and the content of calcium is above the optimal for the growth of the animal. The bones of the animals which were fed on strontium have a deeper metaphysis, but not so great enlargement laterally of the ends of the long bones. The condition resulting from feeding the strontium-containing ration is in effect an exaggerated form of rickets.

The bones of Lehnerdt's animals showed a different condition, in which the epiphyseo-diaphyseal junctions were normal and the marrow cavity was almost or quite obliterated by numbers of small osteoid trabeculæ. This condition,—"strontium sclerosis"—he believed to be closely related to the sclerosis of the bone, which Wegner described as resulting from administration of large amounts of phosphorus. We have been able to reproduce a similar picture by feeding animals on diets with a very high phosphorus content and a low content of calcium. Reference to Lehnerdt's diet will show that the basal ration (muscle meat and fat) was very low in calcium, but contained a very large amount of phosphorus. Moreover, the strontium added to his diet was given as $Sr_2(PO_4)_2$. There can be no doubt that the effect of feeding strontium is influenced by the ratio between strontium and phosphorus in the diet. The same is true in regard to calcium. It is evidently possible, by varying the ratio between phosphorus and either of the two alkaline earths, to produce either a condition which cannot be differentiated from rickets, or an osteosclerosis. The sclerosis obtained by Lehnerdt caused by strontium resembles the sclerosis which follows high phosphorus feeding, because it is the same condition. Sclerosis is not caused by strontium, phosphorus or calcium, but by a salt mixture in which the Ca ion is low as compared with the phosphate ion. In so far the behavior of calcium and strontium are alike, but although strontium is stored in the bone it cannot be made to serve the purpose of the lime salt. Thus, the administration of cod-liver oil to animals whose calcium has been replaced by strontium does not cause any change in the pathological picture produced.

CONCLUSIONS

1. When strontium replaces calcium in an otherwise satisfactory diet (2.2 per cent of strontium carbonate), it stimulates growth, and causes the bones to develop the characteristic picture of rickets "strontium rickets."

2. The anatomical lesions, described by Lehnerdt as induced by the administration of strontium to dogs, were not due to the strontium alone, but to a diet very *poor in calcium* and *high in phosphorus*, and deficient in the factors fat-soluble A or a second dietary essential which we have shown to be associated with certain fats, notably cod-live oil.

3. Cod-liver oil does not enable the animal to compensate for a faulty diet containing strontium.

LEGENDS TO CHARTS

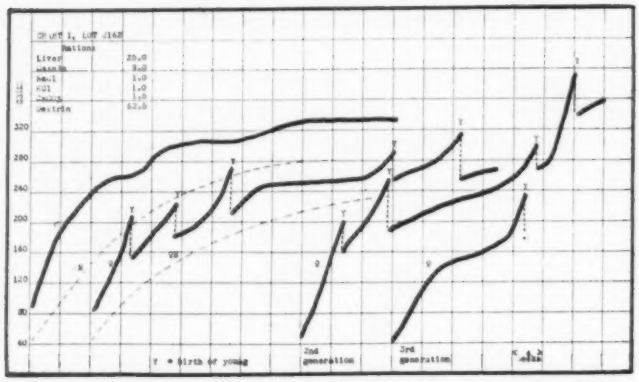


Chart 1. The records of the animals whose curves are shown illustrate the adequacy of the organic components of our diet 2162, which served as the food mixture in our experiments on the effect of strontium feeding on the histological structure of the skeleton. When calcium was added to this mixture, as in diet 2162, to the extent of 1.5 per cent, optimal growth and very good nutrition were secured over a long period. The 25 per cent of liver furnished all the fat-soluble A and water-soluble B which the animals required both for growth and repeated reproduction. Whatever content of the calcium depositing factor it contained was likewise derived from the liver. Their skeletons were of good quality, and were histologically normal. Growth curves for three generations are shown in the chart. This food mixture without the calcium addition is incapable of inducing any appreciable amount of growth (see Chart 2, Lot 2581).

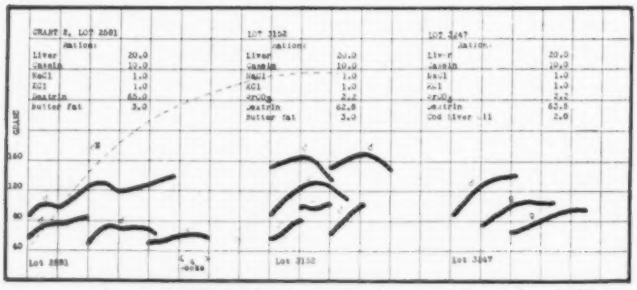


Chart 2. Lot 2581 had the same diet as that of Lot 2162 except that calcium was omitted. Three per cent of butter fat was added in its place. This diet was of very high quality except for its deficiency in calcium. A suitable addition of calcium converts it into a food mixture capable of inducing excellent growth and of maintaining a family of rats in good nutritive condition through at least three successive generations. These rats did not develop typical rickets, but the histological picture presented by their bones bore a fundamental resemblance to this disease. The description of sections of their bones will be found elsewhere in this paper.

Lots 3152 and 3247 had a diet like that of Lot 2581 except that strontium carbonate was added in amount sufficient to give the atomic equivalent of strontium for the calcium in 1.5 per cent of calcium carbonate. Lot 3152 received 3 per cent of butter fat and Lot 3247 received 2 per cent of cod-liver oil. The animals in both groups developed typical rickets.

It is of great interest that strontium stimulated osteoid production to such a marked degree. It led to the formation of the typical rachitic metaphysis, notwithstanding the presence of a great abundance of the substance furnished by cod-liver oil. This substance in the presence of strontium, under the defective nutritive conditions which are maintained by the diet administered to Lot 2581, failed to protect the animals against the pharmacological effects of strontium. It is further of interest to note that under the experimental conditions here described, cod-liver oil is no more effective than is butter fat in exerting a directive influence on bone development.

EXPLANATION OF PLATES

Fig. 1. Photomicrograph of the lower end of the femur of a rat, to show the lesion which follows the substitution of strontium for calcium in the diet. Note the wide metaphysis and the irregularity of the epiphyseal cartilage. The line formed by the rests of old calcified intracellular substance (r) across the bone at the junction of the shaft with the metaphysis marks the situation of the growing area where the effect of the strontium first became apparent. Note also the overgrowth of osteoid tissues, the osteoporosis of the shaft, and the normal medullary cavity.

Leitz micro-summar objective, 35 mm.—no ocular.

Fig. 2. To show the degeneration of the cartilage in the neighborhood of the blood vessels.

Leitz No. 6 dry objective—no ocular.

Fig. 3. Same magnification as in the preceding picture. The picture shows proliferation of the cartilage cells with resulting reduction in the size of the individual cells. From an area in which cartilage cells were undergoing metaplasia under the influence of the blood vascular system.

Fig. 4. High power photograph showing the osteoid tissue. Note the small size of the nuclei of the osteoid corpuscles and the lamellar arrangement of the osteoid.

BIBLIOGRAPHY

1. Lehnerdt, Friedrich: Zur Frage der Substitution des Calciums im Knochensystem durch Strontium. Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie, 1910, XLVII, 215.

2. Stoeltzner, H.: Ueber den Einfluss von Strontiumverfutterung auf die chemische Zuzammensetzung des wachsenden Knochens. Biochemische Zeitschrift, 1908, XII, 119.

3. McCollum, E. V.; Simmonds, N.; Shipley, P. G.; and Park, E. A.: Studies on Experimental Rickets, VI. The Effects on Growing Rats of Diets Deficient in Calcium. Amer. Jour. of Hygiene, 1921, I, 492.

4. Park, E. A.; Shipley, P. G.; McCollum, E. V.; Simmonds, Nina: The Effect of Diets Very High in Phosphorus and Very Low in Calcium on the Development of the Bones in Young Rats. Proceedings of the American Society of Biological Chemists, 1921, v. 7.

5. McCollum, E. V.; Simmonds, Nina; Shipley, P. G.; and Park, E. A.: Studies on Experimental Rickets, VIII. The Production of Rickets by Diets Low in Phosphorus and Fat-soluble A. Jour. Biol. Chem., 1921, XLVII, 507.

6. Shipley, P. G.; Park, E. A.; McCollum, E. V.; and Simmonds, Nina: Studies on Experimental Rickets, XVIII. Is There More than One Kind of Rickets? Amer. Jour. Dis. of Children, 1922, XXIII, 91.

FIG. 1.

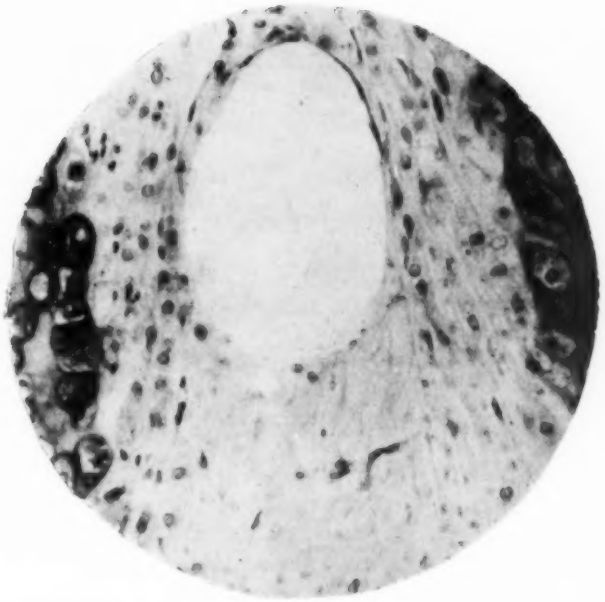


FIG. 2.

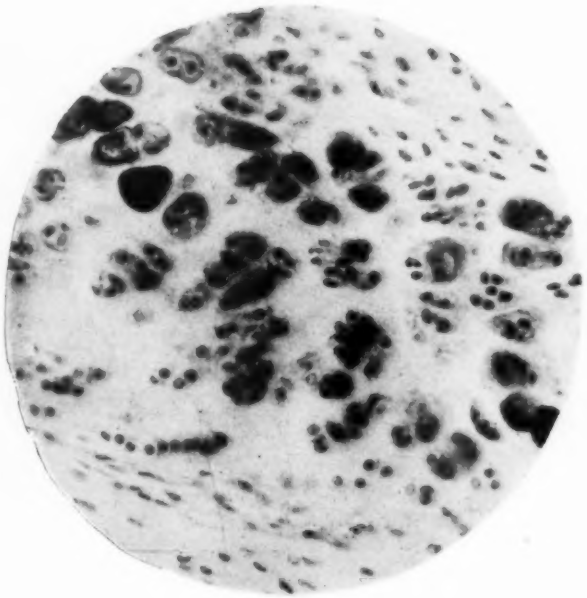


FIG. 3.

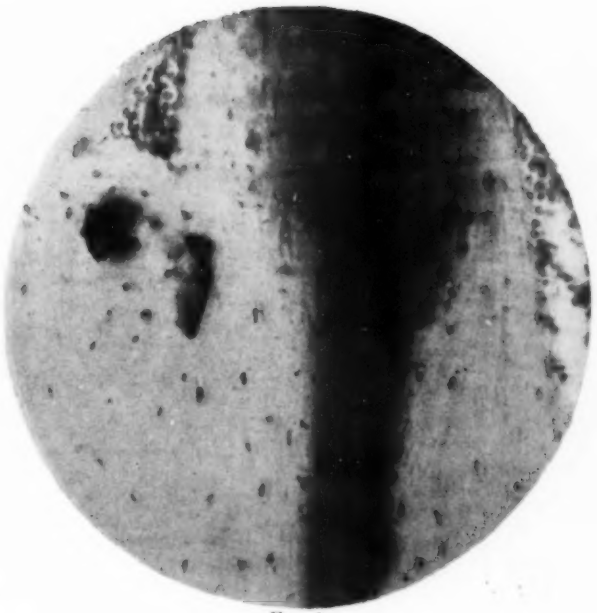


FIG. 4.

CARCINOMA OF THE CERVIX UTERI*

A VERY EARLY CASE

By KARL H. MARTZLOFF

(From the Department of Gynecology of The Johns Hopkins Hospital and University)

The case reported is one of very early carcinoma of the cervix. A panhysterectomy was performed and today the patient feels perfectly well. Of the ultimate result, however, nothing can as yet be said with certainty.

The patient, Mrs. M. M. (Gyn. No. 27080-27165) is a white woman, widowed, 55 years of age, who has had two normal pregnancies and two miscarriages. Her menopause occurred five years ago.

Three months before coming to The Johns Hopkins Hospital the patient began to have a profuse, watery, vaginal discharge which soon became blood-tinged and malodorous. On admission to the hospital, August 13, 1921, examination revealed nothing grossly abnormal. Nevertheless, the history of a foul-smelling, blood-tinged, watery, vaginal discharge commencing five years after the menopause was so suggestive of malignancy that it was decided to perform a diagnostic dilatation and curettage.

When the cervix was dilated, a small quantity of purulent material escaped, evidently from the uterine cavity. Curettement brought away a very small quantity of tissue, and not the large amount usually obtained in definite cervical cancer. Frozen sections made at the time of this operation showed nothing that could positively be identified as cancer, so the patient was returned to the ward. Celloidin sections made on the following day, however, showed tissue which we considered undoubtedly malignant, though by no means giving the picture of a fullblown cancer (Figs. 1 and 2).

The patient at that time refused a radical operation, and left the hospital, only to return in two weeks, when a modified Wertheim operation was done. Her convalescence was uneventful and she is now apparently well.

On macroscopic examination, the pathological specimen with its apparently normal cervix was, to say the least, depressing, for we felt that through a grave error in diagnosis on our part the patient had been subjected to a serious and unnecessary operation. Figure 3.† is a photograph of the specimen in longitudinal section and tells graphically why, so far as could be seen, we were in the face of a surgical success but a diagnostic failure.

Pathological Examination: (Gyn. Path. No. 27099).—The specimen consists of the entire uterus with its adnexa and a liberal margin of vaginal mucosa. The uterus measures 7 x 4 x 3.5 cm. The vaginal cuff measures from 1 to 1.5 cm. in length and is normal in appearance. The lips of the cervix and the cervical canal are pale grey, smooth and glistening, and present nothing noteworthy. The uterine cavity is dilated, its walls are somewhat irregular, of a dull, dirty, grey color, and scraping brings away nothing in the nature of an exudate or a necrotic mambrane. Blocks are taken from all portions of the cervix, uterus, parametrium, and adnexa.

Sections of the uterus show the myometrium infiltrated with numerous round and occasional polymorphonuclear leucocytes. No endometrium is present. The only other sections of interest are those taken from the area indicated at "A" (Fig. 3) on the anterior cervical wall. Here the cervical epithelium proximal to "A" has for the most part its usual appearance, but in certain areas it shows a definite departure from the normal. In Figure 4 both the normal and abnormal stratified cervical epithelium is seen. At *x* the epithelium is hyperplastic and shows a well defined metaplasia in which the epidermal cells have lost the normal gradation from stratum mucosum to stratum germinativum. Instead, one sees large oval and broad spindle-shaped cells rather uniform in size, with hyperchromatic nuclei which show some irregularity in staining reaction. There are some nucleoli, and mitotic figures (Fig. 5, *a*) are not uncommon. These cells have practically no eosin-staining cytoplasm.

Just distal to "A" (Fig. 3.) sections show cell-nests (Fig. 6.) in which the cells resemble those just described, although here the irregularity in size, shape, and staining reaction is more marked and the mitotic figures are more numerous (Figs. 7 and 8.).

We believe that this is an early carcinoma of the cervix uteri of a type which we have been seeing rather frequently of late and concerning the nature of which competent pathologists are not in accord.‡

Because of the difference of opinion which occurred after the reading of this paper, we were glad to follow

* Read before the Medical Society of The Johns Hopkins Hospital, November 7, 1921.

† This photograph was retouched in order to bring out more distinctly the smooth surface of the mucosa lining the vaginal cuff and cervix.

‡ In this connection it is only fair to state that when the sections represented in Figs. 1 and 6 were submitted to four well known pathologists, three considered the tissue to be cancerous and one did not consider the evidence sufficient to warrant such a diagnosis. However, after seeing the serial sections represented in Fig. 9, the opinion in favor of cancer was unanimous.

Dr. MacCallum's suggestion and make serial sections of the entire cervix, in order to find, if possible, some areas of more advanced carcinomatous invasion. Serial sections made from the right half of the cervix, from which the original blocks were taken, show a condition quite similar to that which has just been described. Serial sections from the left half of the cervix show cell-nests (Figs. 9,*b* and 10) similar to those represented in Figure 6, but in addition there are areas of actual lymphatic invasion (Figs. 9,*c* and 11), small clumps of cancer cells lying free in what appear to be endothelial-lined spaces. The microscopic invasion in none of these sections goes beyond a depth of 2 mm. below the surface of the cervical canal and the entire neoplastic process is limited to the anterior portion of the cervix. This, we feel, proves conclusively that our case is one of a true malignant neoplastic process and also demonstrates the value of making serial sections, in disputable cases, when numerous routine sections do not furnish the information desired.

Discussion

Dr. Thomas S. Cullen: "Dr. Martzloff's squamous-cell carcinoma is evidently a very early one. We have never found nuclear figures in the squamous epithelium of the cervix except in cases of carcinoma of the cervix."

After discussing briefly an early squamous-cell carcinoma of the cervix, which he had reported in *Surgery, Gynecology and Obstetrics*, in August, 1921, Dr. Cullen added: "We fully realize that nuclear figures do occur in the squamous epithelium, otherwise there could be no reparation when defects occur. But they are so uncommon that we have not encountered them in our routine work except where malignancy exists. We have also failed to note them in the cylindrical epithelium of the cervix.

"On the other hand, nuclear figures are regularly met with in the epithelium of the body of the uterus, and in cases of hyperplasia of the endometrium nuclear figures are frequently found in the stroma cells of the mucosa."

THE CAPILLARIES OF THE BONE MARROW OF THE ADULT PIGEON

By Charles A. Doan

(*From the Anatomical Laboratory, Johns Hopkins University*)

Since the time when it first became known that the red marrow of the long bones gives rise to certain of the essential cells of the circulating blood in vertebrates, the question as to the exact derivation and relationship of the different types of blood-cells to each other has proved most puzzling and fascinating to scientific investigators. To the clinician, also, the question has been, and is today, a most urgent one, upon the understanding of which will depend the treatment of certain well-recognized but as yet little-understood diseases.

This eagerness to understand the origin and development of the blood elements *per se* has relegated the question of the circulation and exact vascular pattern of the marrow to a position of minor significance, though its importance, at least for the delivery of these cellular elements, once formed, to the organism as a whole, is quite apparent. With but few exceptions, the majority of workers in the field have confined their efforts to the main issue of cellular derivation and development and have either ignored altogether or considered only incidentally and superficially the vascularization of bone marrow.

Three theories as to the nature of the circulation in adult marrow have been advanced, no one of which, however, has been established convincingly enough to be generally accepted, though all three have received more or less support. Rindfleisch [1] represents one extreme. He believed that his experiments showed that the blood spaces are lined by parenchyma alone and have no endothelial walls. Langer,[2] on the other hand, believed the marrow to be supplied by an entirely closed vascular system. Bunting [3] hypothesized vessels lined with endothelium but with openings at various points communicating directly with the medullary parenchyma. Drinker [4] tended to support this view, though he expressed some doubt of the conformity of normal adult marrow to this pattern.

The greatest progress made toward solving the problem of the origin and evolution of individual types of blood-cells has been through embryological studies. Probably the most representative work on the embryology of the blood is that carried out by Danchakoff [5, 6] and Sabin [7, 8] on birds and by Maximow [9, 10] on mammals. Both Maximow and Danchakoff recognized the relationship between endothelium and blood-cells, not only in the stage of the primitive blood-islands but also in somewhat later stages; both have thought, however, that endothelium gives rise only to indifferent blood-cells. Schridde,[11] on the other hand, has described the direct transformation of endothelium into erythroblasts in early human embryos. Maximow believed that although the early erythroblasts of mammalian embryos are intravascular in origin and derived indirectly from endothelium, the ultimate erythroblasts are a group of cells extra-vascular in origin. This question has now, however, been reopened by the work of Sabin,[7] and it was not until she had

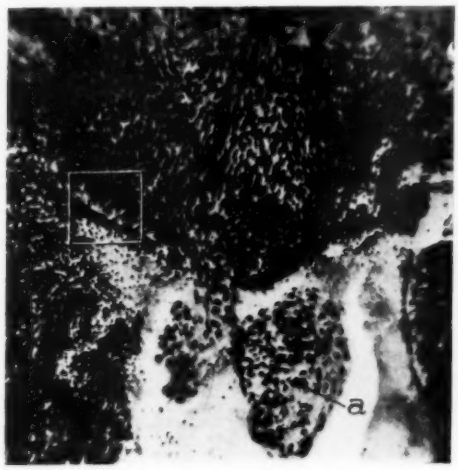


FIG. I.

FIG. 1.—(Gyn. Path. No. 27069.) Section of curetting showing the hyperplasia and metaplasia. At *a* is a multinuclear giant cell. There are also mitotic figures, see Fig. 2.

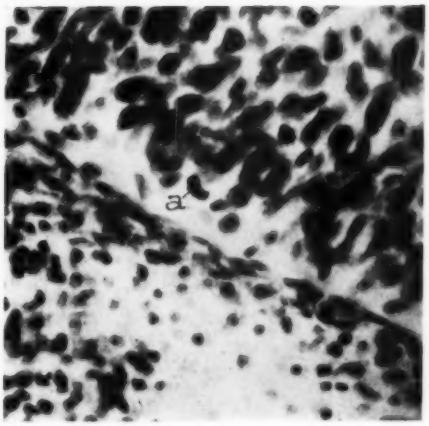


FIG. II.

FIG. 2.—(Gyn. Path. No. 27069.) A high power picture of the blocked area in Fig. 1. At *a* is a well defined mitotic figure in its metaphase. The irregularity in size, shape and staining reaction of the cells is also well seen.

FIG. III.

FIG. 3.—(Gyn. Path. No. 27099.) Showing smooth cervical lips and vaginal mucosa. The uterine cavity is dilated and was filled with purulent material. At "A" is the situation of the carcinoma which is not visible on macroscopic examination.

FIG. IV.

FIG. 4.—(Gyn. Path. No. 27099.) Low power photomicrograph from (Fig. 3) "A." At *x* is seen the epithelial hyperplasia which even under the low power reveals a metaplasia. Here the cells have lost the normal transition stages of the gradation from stratum mucosum to stratum germinativum.

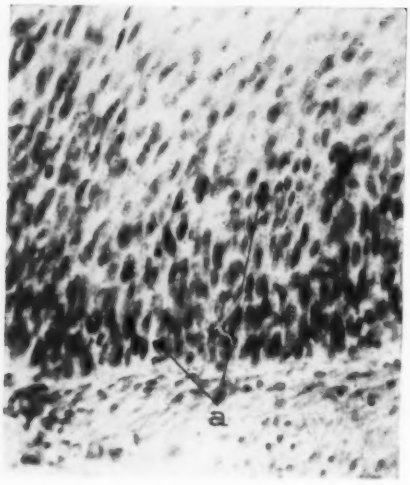


FIG. V.

FIG. 5.—(Gyn. Path. No. 27099.) A high power picture of the area enclosed in square in Fig. 4. At *a* are two mitotic figures, one in its metaphase and the other in its anaphase.

FIG. VI.

FIG. 6.—(Gyn. Path. No. 27099.) Low power photomicrograph of area just distal to "A" in Figure 3. The areas at *x* show typical carcinoma, the innermost nest of cells lying 1 mm. beneath the surface of the cervical canal.

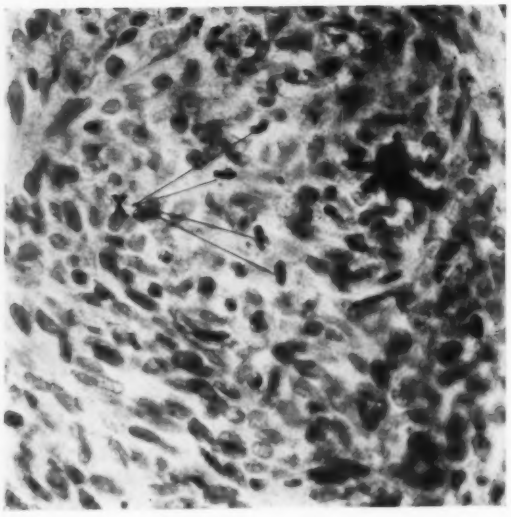


FIG. VII.

FIG. 7.—(Gyn. Path. No. 27099.) High power picture of area inclosed in square in Fig. 6. The mitotic figures are well seen at *x*.

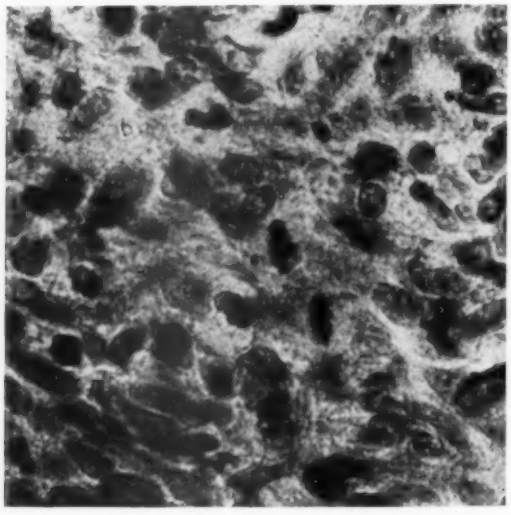


FIG. VIII.

FIG. 8.—(Gyn. Path. No. 27099.) A higher power picture of Fig. 7 area *x*, showing the metaphase stage of mitosis in three of the cells.

FIG. IX.

FIG. 9.—(Gyn. Path. No. 27099. Serial No. 60.) A low power picture of a section from left half of cervix. *a* shows cervical canal. *b* is a cell nest. *c* points to three cell nests in the anterior lip of cervix, 2 mm. from surface of the cervical canal.

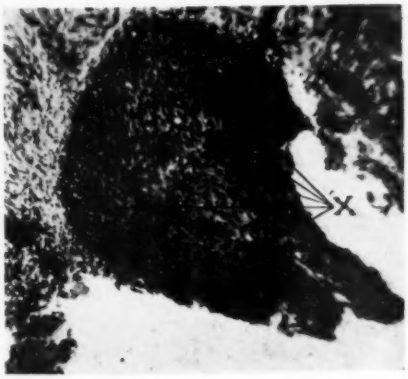


FIG. X.

FIG. 10.—(Gyn. Path. No. 27099.) A high power of *b* Figure 9. Numerous mitotic figures are seen at *x*. Under higher magnification the appearance is practically identical with that seen in Figures 7 and 8.

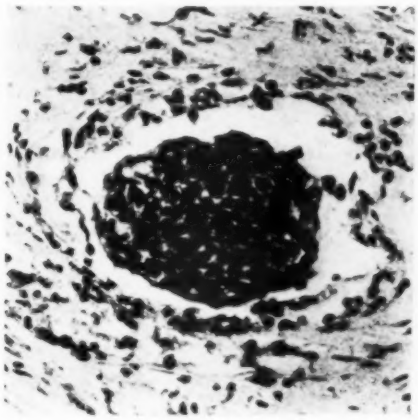


FIG. XI.

FIG. 11.—(Gyn. Path. No. 27099.) A high power picture of area inclosed in square in Figure 9.

actually seen, by direct observation on living chick embryos during the second day of incubation, the differentiation of the red cell from early endothelium, and later [6] the origin of the monocyte cell-series and clasmatocytes from the same source in chicks on the third and fourth days, that the etiological importance of the endothelium and hence the significance of the exact pattern of the vessels of the marrow of the mature organism was adequately emphasized. Stimulated by this work and its potential relationship to the production of blood elements in the functioning adult organism, certain questions of interest naturally arose. What is the extent and distribution of the endothelium in the adult marrow? Is the vascular system a closed one? Are the connecting vessels between the arteries and veins large sinusoidal spaces leading directly from arterioles to venules, or is there a capillary bed? If there is a definite capillary bed, how extensive is it and how distributed? Are all of the channels patent at all times or, in emergencies, is there an increase in the vascular bed? As already indicated, the information, concerning these points, to be obtained from the literature is quite unsatisfactory. More definite answers to these questions and a more thorough understanding of the architecture of bone marrow attendant upon them should afford the rational basis upon which to proceed with a further analysis of the relationship of the blood-elements to the endothelium in marrow.

This problem was begun at the suggestion of Dr. F. R. Sabin, and it is a pleasure to acknowledge here her continued interest and assistance throughout its development.

Material and Method

The conclusions reached in this paper are based largely on a series of investigations on about forty adult pigeons. Further experiments of a similar character conducted on the cat, rabbit, and white rat seem to substantiate and corroborate the gross findings in the pigeon, though a larger series will be necessary for complete corroboration in the mammal.

The attempt was made to get complete injections of the vascular system of the bone marrow; india ink, one part, in physiological saline, three parts, proved to be the best medium for injections. My most successful injections were obtained with a pressure of 130 mm. of mercury. When the pressure was materially increased above this point, rupture and extravasation frequently occurred. With pressures below this point incomplete injections resulted.

The radius and ulna of one side were fixed and cleared by the Spalteholtz [12] method, with the marrow *in situ*. The "marrow pencils" were removed from the bones of the opposite side, fixed in Helly's fluid at 38°C, dehydrated, cleared and embedded for sectioning, celloidin embedding proving better for the study of the individual cells, as pointed out by Maximow and Danchakoff. The humerus in the pigeon has no bone marrow, in fact it is a pneumatic bone.

Observations

In the earlier incomplete injections the gross architecture of the bone marrow is plainly evident in the cleared specimens. The medullary artery enters the marrow cavity near the center of the diaphysis, perforating the compact tissue obliquely, and immediately divides into the main branches which diverge abruptly, one extending toward each epiphysis. These two main arterial trunks divide in turn about half way to the epiphyses and extend from the point of their origin to the end of the marrow.

In addition to this main arterial supply, there are to be seen numerous small vessels entering along the shaft of the bone, primarily to nourish the cancellous and compact tissues, but anastomosing at the periphery with the arterioles of the central vessels. Near the epiphyses, one or two small arteries are usually seen entering the marrow cavity through the bone. There is frequent and intimate intercommunication along the entire shaft between the nutrient vessels of the Haversian canals and the circumferential end-arterioles and venules of the medulla of the bone. These anastomoses form a very striking picture in a cleared specimen and give a new insight into the delicacy of the vascular interlacings and the extent of their ramifications. We are not dealing with two more or less separate and distinct systems, one to nourish the marrow and the other cancellous and compact tissues, but with one interdependent and communicating system.

There are three groups of veins in the long bone: (1) From one to four parallel veins accompany the artery and traverse the shaft from each end, to unite near the center in a single efferent vein. Thus there seems to be a group of main central vessels which arise from the nutrient artery and corresponding vein and extend the full length of the bone from a zone near the proximal almost to the distal epiphyseal line. (2) Several large veins emerge near the articular extremities from the extensive vascular area of red marrow, always more prominent toward the epiphyses. (3) There are numerous small veins along the diaphysis, which drain the compact tissue and the peripheral area of the marrow, and, with the small nutrient arterioles of the shaft, form the abundant vascular network of the periosteum.

This general vascular pattern holds for both the radius and ulna of the pigeon, the individual bones differing only in the number of their central vessels in direct relation to their relative size and the extent of bone marrow to be vascularized. In relatively complete injections, the central vessels cannot be seen from the surface in even the most perfectly cleared specimens, so dense is the network of injected vessels, as will be indicated later.

Specimens showing the next stage of a partially complete injection illustrate again the gross picture observed in lesser injections of the marrow cleared *in situ*. The central vessels are still visible and it may be seen that there are smaller branches coming off at an angle from the main arteries, which extend toward the circumference. These begin almost at the center of the shaft but become more numerous and dense toward the ends. At each epiphysis there is a veritable spray-like shower of fine vessels which ramify to every part of the marrow, and supply the epiphyses as well, but stop abruptly at the line of cartilage which forms the articulation of the joint. The characteristic vessels of embryonic cartilage have disappeared in the mature state.

The artery and its branches are easily distinguishable from the veins by virtue of their smaller calibre, firmer wall, and less tortuous course, and also by the fact that the lumen is more closely packed with the particles of carbon. The divisions of the artery are characteristic; the branches come off at an acute angle and the subdivisions are much less numerous than those of the corresponding veins. The arterioles at the periphery are characteristic in their delicacy, scarcity, and apparently limited distribution.

The venous system presents a similar picture though greatly magnified and multiplied. Coming off from the central vessel, almost at right angles, are the large distended veins which at once branch outward toward the circumference in an ever-widening balloon-shaped bed to anastomose eventually with branches from tufts on either side. The large calibre of the vessels is strikingly maintained and though there is some decrease in the lumen toward the periphery it is not commensurate with the extent of the branching. The most apparent and striking thing about the entire vascular system of the bone marrow, both in gross and in microscopic view, is this extensive venous ramification and its very evident capacity for large quantities of blood.

A still better comprehension of these venous and arterial tufts and the means by which they become continuous with each other is obtained from a more complete injection, in sections 100 to 150 microns thick. In such preparations can be plainly seen what I have termed the "transition capillaries" leading directly from the arterioles to the venous sinusoids and apparently with very little true arterial capillary bed. This patent capillary bed connecting arteries and venules is extremely circumscribed and it is not until the venous sinusoidal anastomoses are reached that the blood spreads out in lacing and interlacing vessel tufts, thence to be directed from the tuft-like branchings into larger and larger vessels, eventually to enter the central longitudinal vein almost at right angles, or to find egress by way of one of the other venous outlets. The marrow appears to be divided almost into segments or lobules by these venous tufts, so completely do they ramify in definite areas, anastomosing on all sides with the ramification of bordering tufts. The relationship of the arterial tree to the venous tufts on either side and the capillary transitions from one to the other, even though not extensive, are easily distinguishable and very characteristic. There is little doubt that these extensively distributed, spacious, thin-walled venous sinusoids form normally the real functioning vascular bed of the marrow. These are the vessels which have been seen and described as the fundamental units of the bone marrow by those who have written in this field. By most workers they are termed the *venous capillaries*. It would seem that *venous sinus or venous sinusoid* might be the more appropriate and desirable terminology, inasmuch as there are already two types of true capillaries in the marrow as recognized and interpreted in these observations.

All of the vessels thus far described are plainly apparent, either grossly or with the aid of the binocular microscope. The analysis of the circulation up to this point has been comparatively simple, but it was when an attempt was made to study under the oil immersion, the detailed ramification of the smaller vessels, the extent and continuity of the endothelium, that difficulties were encountered. It was soon found that analysis of these finer points in a normal marrow would be very unsatisfactory and impracticable. In order to analyze with any certainty the finer ramifications of the vascular pattern, it is essential in the first instance, at least, to have a marrow depleted, as far as possible, of all the free cells. An attempt was therefore made to produce experimentally a hypoplastic bone marrow in the pigeon. The desired condition was secured through simple starvation for a period varying from ten to eighteen days.

In such a hypoplastic marrow three types of cells were observed—fat cells, reticular cells, and endothelial cells. In order to analyze the relations of these three cell types the vessels of the marrow were washed out with salt solution and then injected with India ink. The fat cells, together with their nuclei, were readily distinguishable and quite characteristic. They were more numerous in the hypoplastic marrow, having replaced apparently to a large extent the depleted cellular areas. In the fixed tissue these cells appear as empty spaces, limited by a thin but distinct membrane; and each contains a more or less flattened oval nucleus, eccentrically placed, and but faintly stained, owing to the small amount of chromatin. Such cel's make an easily discernible network. Frozen sections of the fresh tissue, stained with Sudan III, indicate the increased extent of these deposits of fat in the depleted marrow.

Reticular elements which conformed to all of the known criteria were to be seen. They were large pentagonal or hexagonal cells with large, round vesicular

nuclei; the cytoplasm took a faint eosin stain, the nuclei showed a moderate content of chromatin.

The endothelial cells in the main conformed to certain standards and were recognized through various characteristics. In the areas where the endothelium could be seen lining the venules and the capillaries connecting them, there was no difficulty in its identification, but there are capillaries in the bone marrow where, even after taking all the histological characteristics of endothelium into consideration, the cells can not be definitely classified in normal uninjected marrow. Unfortunately, a specific stain for endothelium has not been developed up to the present time and such characteristics as size, morphology, and peculiarities of the nuclei are not adequate criteria. Realizing fully, then, the difficulties of final determination in the case of a certain few individual cells, I have tried to analyze the picture presented by these injections on the basis of the data available at the present time for their interpretation, while recognizing the limitations of our methods.

It is not until sections as thin as 5μ (Figs. 1 and 2) from a relatively complete injection of a hypoplastic marrow, are seen under the oil-immersion that the full import of the nature and extent of the bone marrow vascularization begins to be realized and understood. First of all, the gross structures—the main longitudinal vessels, transverse smaller branches, arterioles, a few transition capillaries, and venous sinusoids described above—are easily verified in the serial sections. But in addition to these, I have found, appearing between the fat spaces in well-outlined and clearly defined channels, a most extensive system of capillaries, hitherto unsuspected. Many of these capillaries appear to have been non-patent and functionally dormant so far as the circulation is concerned. They are collapsed so that only a trace of fine ink granules reveals the presence of a potential lumen, the calibre of which appears insufficient for the passage of even a single blood-cell element without difficulty. In an ordinary injection they are totally collapsed and are seen as septa surrounding the fat cell spaces. Toward the epiphyses there is a complete encircling of each fat space by these channels. They are seen to lead directly from the large venous sinusoids via typical conical openings and appear to be continuous with them. There is no break in the continuity of the endothelium which forms these slender channels from sinusoid to sinusoid. There is no extravasation at any point and the material injected follows these vessels everywhere. It is evident that these channels are closed in the sense that there is no extravasation or diffuse permeation of the tissue by the injected ink.

The attempt to differentiate an extravasation from a true circumscribed distribution of perfused particles within definite channels was not made without a full appreciation of the marked tendency of such granules to follow a reticular framework closely in any injection into diffuse connective tissue. This characteristic of reticular tissues to be outlined by extravasated particles, thus simulating, more or less, definite channels, is recognized and acknowledged, and it is obvious that the possibility of error of interpretation in injections of mesenchymatous tissue requires a corresponding amount of attention and care in analysis.

There are, however, five points apparent in the interpretation of these studies which emphasize strongly the non-fenestrated character of the vascular bed of the bone marrow: (1) In injections showing a diffuse permeation of the medullary parenchyma there have been demonstrable ruptures in vessel walls. (2) In extravasation it is clear that the extruded granules are neither phagocytized nor regularly distributed along one side, but adhere promiscuously and heterogeneously to the surface of the parenchymal cells, thus more or less concealing their outline. In contrast to this, the particles within a definite lumen are scattered here and there along the sides of the lining cells on the inside of the channel only. (3) In an analysis of comparatively complete injections, showing this extensive, intersinusoidal capillary bed, not only can these channels be distinctly followed by the granules of ink, but the reticular network or framework of the medullary parenchyma can be seen in the same areas without any granules of ink attached thereto. (4) The walls of the veins and venules appear as continuous endothelial-lined channels, similar in appearance to the vascular bed elsewhere in the body, but with conical openings into the tiny capillary network. (5) Finally, I have gotten relatively complete injections of these very fine, extensive, lace-like vessels without the slightest evidence of any of the injected particles outside the closed channels in the parenchyma. In other words, there is no evidence of any fenestrated vessel wall, similar to that described by Mollier[13] for the spleen, in the adult bone marrow studied here. One only need contrast a true extravasation with one of these injections to recognize the difference at once. It is, however, very possible that, in an injection of normal bone marrow which filled only arterioles, transition capillaries and venous sinusoids, these conical capillary openings might be interpreted as fenestrated membranes.

The endothelial cells are thinned out, in contrast to their number and arrangement in a larger vessel, and in many instances have been forced apparently into the interstices between encroaching fat cells and look more nearly like the primitive embryonic endothelium. They can, nevertheless, be seen to line these spaces through which granules of the injected fluid have been forced. The picture, then, is that of a very extensive capillary bed which simulates in the appearance, distribution, and arrangement of its vessels and cell elements, an embry-

onic plexus rather than the ordinary mature capillary plexuses elsewhere recognized in the adult. This plexus is lined everywhere with endothelium.

Summary

1. The arterial supply of bone marrow is secured via the medullary artery, the periosteal vessels along the shaft, and some vessels near the articular extremities which supply the epiphyses as well. The arterioles are relatively few in number.

2. Normally there are a few "transition capillaries" functioning as the intermediary communication between arterioles and venous sinusoids.

3. The very extensive distribution of large-lumened, thin-walled venous sinusoids, probably forming the real functioning vascular bed of the marrow, is the most characteristic thing about the gross circulation in bone marrow. The venous drainage is threefold, corresponding to that of the arterial supply.

4. A hypoplastic marrow is essential for the analysis of the finer distribution of the blood channels. In such a marrow can be seen a very extensive inter-sinusoidal capillary plexus, hitherto unsuspected, its normal state being possibly one of collapse.

5. The vascular system of the bone marrow is a closed system, no fenestrated vessel walls being demonstrable in this series of experiments.

6. Endothelium apparently forms a continuous lining throughout the vascular ramifications in the marrow, being therefore much more extensively distributed through the medium of the wide spread capillary plexus than has been indicated in the usual marrow injections heretofore described.

This report presents one of the active phases of the present day bone-marrow problem. The complete communication, with review of the literature, technic employed, and a series of plates illustrating the findings is being prepared for the Contributions to Embryology, Carnegie Institution of Washington.

FIGURE LEGENDS

Fig. 1.—Drawing of a hypoplastic bone marrow, injected with India ink, showing venous sinusoids and the inter-sinusoidal capillaries; from the radius of an adult pigeon (19A): *e.c.*, endothelial cells lining the capillaries; *r.c.*, reticular cells of the marrow; *n.f.c.*, nuclei of the fat cells; *r.b.c.*, red blood cell; *v.s.*, venous sinusoids; *cap.*, inter-sinusoidal capillaries surrounding the fat cells, with the granules of carbon of the injection fluid scattered throughout the extent of their channels. These capillaries are seen to be in direct communication with the large venous sinusoids. H. & E., 5μ, x 700.

Fig. 2.—Photomicrograph of the same area in the bone marrow, the detail of which is given in Fig. 1. 5μ, x 700.

BIBLIOGRAPHY

1. Rindfleisch, G. E.: Ueber Knochenmark und Blutbildung. Arch. f. mikr. Anat., Bonn., 1880, XVII, 1-11 and 21-42.

2. Langer, K.: Ueber die Blutgefässe der Knochen des Schädeldaches und der harten Hirnhaut. Denkschr. d. k. Akad. d. Wissensch. Math. naturw. Klasse, Wien, 1877, XXXVII, 217-240.

3. Bunting, C. H.: The regulation of the red blood-cell supply. Contributions to Medical and Biological Research dedicated to Sir William Osler. Paul B. Hoeber, 1919, II, 824-828.

4. Drinker, C. K.: The pathological physiology of blood-cell formation and blood-cell destruction. The Oxford Med., Oxford University Press, 1920, II, Chapter XV, 509-587.

5. Danchakoff, W.: Untersuchungen über die Entwicklung des Blutes und Bindegewebes bei den Vögeln. i. Die erste Entstehung der Blutzellen beim Hühnerembryo und der Dottersack als blutbildenes Organ. Anat. Hefte, Wiesb., 1908, XXXVII, 473-589.

6. *Idem.:* Untersuchungen über die Entwicklung von Blut und Bindegewebe bei Vögeln. Das lockere Bindegewebe des Hühnchens im fetalen Leben. Arch. f. mikr. Anat., 1909, LXXIII, 117-181.

7. Sabin, F. R.: Studies on the origin of blood-vessels and of red blood-corpuscles as seen in the living blastoderms of chicks during the second day of incubation. Contrib. to Embryol., 1920, IX, 215-262. A Memorial to Franklin Paine Mall. Carnegie Inst. Wash., Pub. No. 272.

8.————Studies on blood. The vitally stainable granules as a specific criterion for erythroblasts and the differentiation of the three strains of the white blood-cells as seen in the living chick's yolk sac. Johns Hopkins Hosp. Bull., Balt., 1921, XXXII, 314-321.

9. Maximow, A.: Untersuchungen über Blut und Bindegewebe. I. Die frühesten Entwicklungsstadien der Blut- und Bindegewebszellen beim Säugetierembryo, bis zum Anfang der Blutbildung in der Leber. Arch. f. mikr. Anat., Bonn, 1909, LXXIII, 444-561.

10. *Idem:* Untersuchungen über Blut und Bindgewebe. III. Die embryonale Histogenese des Knochenmarks der Säugetiere. Arch. f. mikr. Anat., Bonn, 1910, LXXVI, 1-113.

11. Schridde, H.: Die Entstehung der ersten embryonalen Blutzellen des Menschen. Verhandl. d. deutsch. path. Gesellsch., 11 Tag. Dresden, 1907, Jena, 1908, XII, 360-366.

12. Spalteholz, W.: Ueber das Durchsichtigmachen von menschlichen und tierischen Präparaten. 1911, Leipzig, S. Hirzel.

13. Mollier, S.: Die Blutbildung in der embryonalen Leber des Menschen und der Säugetiere. Arch. f. mikr. Anat., 1909, LXXIV, 474-524.

THE JOHNS HOPKINS HOSPITAL BULLETIN

The Hospital Bulletin contains details of hospital and dispensary practice, abstracts of papers read and other proceedings of the Medical Society of the Hospital, reports of lectures, and other matters of general interest in connection with the work of the Hospital. It is issued monthly. Volume XXXIII is in progress. The subscription price is $4.00 per year.

(Foreign postage, 50 cents.) Price of cloth-bound volumes, $5.00 each.

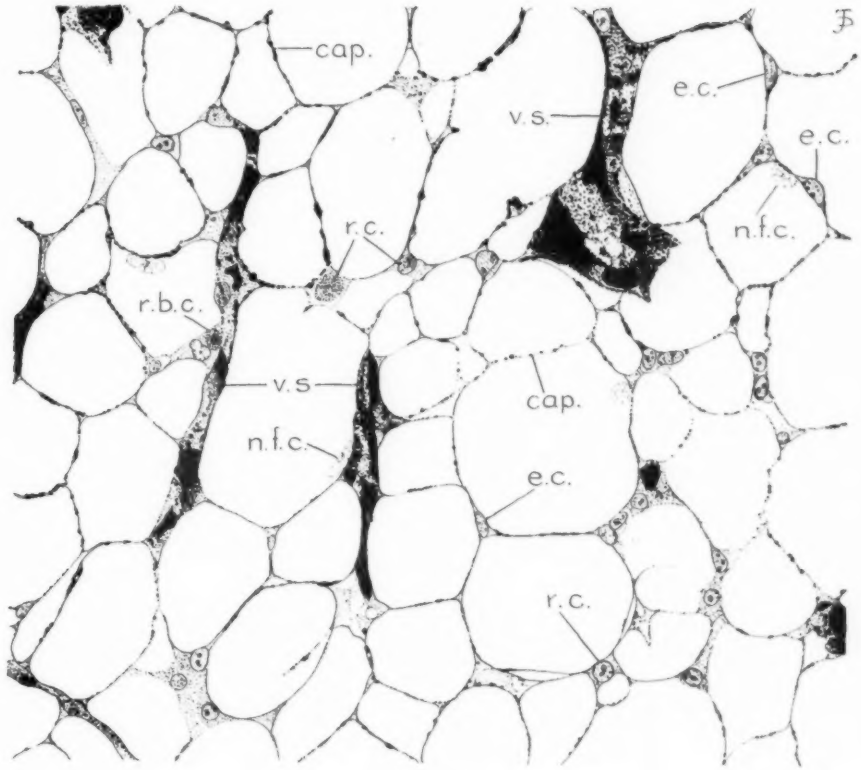


FIG. I.

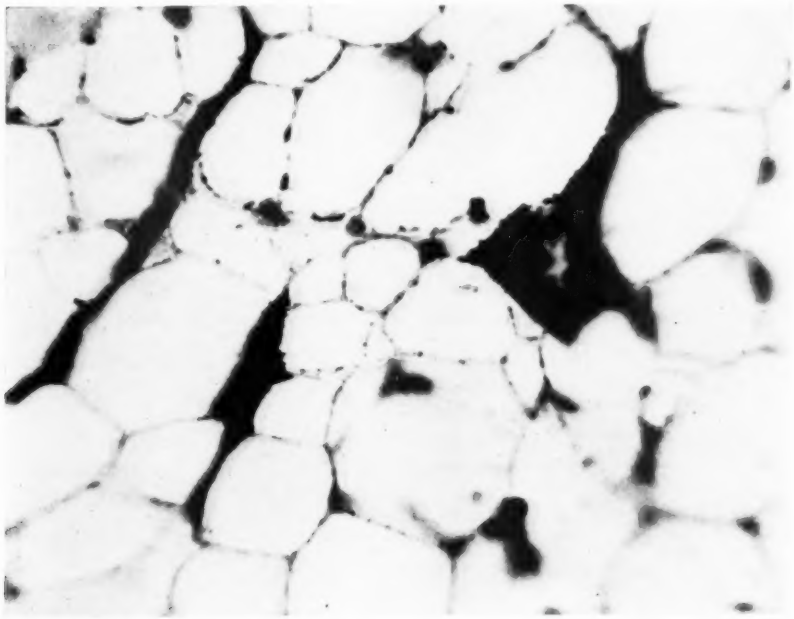


FIG. II.

THE EFFECT OF SLIGHT INCREASE OF TEMPERATURE ON THE BACTERIOSTATIC POWER OF GENTIAN VIOLET

By JOHN W. CHURCHMAN

(*New York*)

That bactericidal agents are more effective if applied hot than if applied cold is perfectly well known, although advantage is seldom taken of this knowledge in practice. From the experimental evidence herewith reported it would seem, however, that *the degree* to which bacteriostatic effects may be influenced by rises in temperature is not sufficiently appreciated.

The organism used in the experiments was peculiarly suited to the purpose, for it was an impeccable gentian-violet-fast strain, the descendant of a single bacterial cell planted on gentian violet agar.[1] This culture did not, therefore, contain the two types of organism (gentian violet positive and gentian violet negative) which I have shown may co-exist in at least some species of so-called "pure cultures" of B. coli.[2]

If gentian violet at room temperature was added to a suspension of this organism and the dye and bacteria allowed to remain in contact for one hour before planting the bacteria on plain agar, the dye was found to be without influence on the growth of the organisms (Fig. 1c). That is to say: this organism—and the same is true of about 90% of all Gram-negative bacteria—was gentian negative, as distinguished from the large majority of Gram-positive bacteria which do not survive exposure to the dye at room temperature, or even at ice temperature (e. g., *B. subtilis*, Fig. 1s).

If the temperature of the dye be slightly raised, however, a different story is told. Figs. 2 and 3, for example, represent experiments in which the strain of *B. coli* under examination has been exposed to stain which is kept at slightly elevated temperatures. At 35° C. the dye was without effect;[3] organisms exposed for 1½ hours to dye kept at this temperature grew as well as if nothing had been done to them (Fig. II).

Heated to 50°, on the other hand, the dye became an efficient bactericide and organisms which had been exposed to gentian violet at this temperature refused to grow when planted on plain agar (Fig. III). That is to say: a bactericidal agent harmless for this strain of *B. coli*, when applied at room temperature, becomes potent if applied at 50°.

That neither of the two agents employed in this experiment—gentian violet and heat—is *alone* capable of bringing about the death of the organism—(capable of this, that is to say, in the degree used in the experiment)—is easily proven. Such an experiment is represented in Fig. IV. The plate marked *A* has been planted with four strokes of bacteria which have been exposed for one hour to gentian violet at room temperature; growth has been uninfluenced by the dye alone. The plate marked *B* has been inoculated with four strokes of an emulsion of the organism which has been kept at 50° C. for one hour; there has been no inhibition of the growth of the organisms due to the heat alone.

DEGREE OF HEAT NEEDED

No attempt has been made to determine the exact temperature at which gentian violet—a dye without effect on this strain of *B. coli*, if applied at room temperature—becomes bacteriostatic. Indeed, certain observations make it seem likely that the lethal dye-heat temperature is not a constant, but varies with the age of the organism used in the experiment and with other little understood factors. It does seem certain, however, that the increase in temperature necessary to effect this change is not very great. In one series of experiments, for instance, exposure to the dye at 30° for 1½ hrs. resulted in the death of nearly all the organisms, though exposure to the dye for the same length of time at room temperature (which, on the day of the experiment, was 26°) was without effect. A result of this kind, with so slight an increase of temperature, could not be constantly obtained; but the experiment indicates how slight an increase of temperature *may* be needed to make this impotent dye potent. 55° for 1 hr., or 50° for 1½ hrs. seems to be constantly bacteriostatic for this strain of *B. coli*—a result which should be compared with that of exposing a Gram-positive organism, like *B. subtilis* to gentian violet at ice temperature: for this Gram-positive organism the dye is bacteriostatic even at these very low temperatures.

EXPLANATION OF THE PHENOMENON

The reason why this slight degree of heat renders an impotent dye bacteriostatic is not altogether clear. It might seem that organisms which survived exposure to the unheated dye, but were killed by the dye when slightly heated, died—in the latter instance—because they were

[1] J. Exp. Med., 1921, XXXIII, 583.

[2] J. Exp. Med., 1921, XXXIII, 369.

[3] This was the case in this particular experiment; as will later appear the results were not constant as to the *exact* temperature at which the dye became potent, and in some experiments even so slight a rise of temperature as this was sufficient to produce the effect.

subjected to two slight insults which (though neither alone was able to kill the bacteria) together were able to bring this result about. That this is not the case may be readily shown. If the organisms be subjected to the dye at room temperature and then to heat (50° for one hour), *in succession*, they grow as readily as if nothing had been done to them. That is to say: they must be exposed to the *heated dye* and not simply to *heat and dye* (Fig. V).

It is well known that the penetrative power of chemical agents is increased by heating them and it is possible that this may explain the potency of heated gentian violet for this strain of gentian-negative *B. coli*. No such increase in penetrative power is, however, demonstrable. In Fig. VI is shown a micro-photograph of organisms which have been stained by exposure to gentian violet for one hour at room temperature, and in Fig. VII organisms which have been exposed to the dye at 50°; if there is any difference in penetrative power of the dye as measured by depth of staining, it is in favor of the unheated dye, for the organisms which had been exposed to it are even darker than those stained with heated dye.

The parallelism between the Gram reaction and the gentian reaction, established in 1912,* might be invoked for light on the phenomenon under discussion. It is, in general, the Gram-negative organisms which survive exposure to gentian violet and the Gram-positive organisms which are killed by it. The strain of *B. coli* used in the experiments described, was, as already stated, an impeccable gentian-negative strain; it was definitely Gram-negative; and it survived exposure to gentian violet at room temperature, as the great majority of Gram-negative organisms do. It did *not* survive exposure to dye heated to 50°. Did this heating fix the dye so that it would be difficult to remove it with alcohol? And did this fixation account for the acquisition of potency by the dye? And do Gram-positive organisms owe their inability to withstand exposure to unheated dye to a similar power of fixation, which is—in their case—expressed by their Gram-positiveness? No such fixation of the dye by heating can be demonstrated; organisms which have been exposed to heated dye are quite as readily decolorized by alcohol—quite as definitely Gram-negative—as those which have been stained with unheated dye.

The difference in behavior of this strain of *B. coli* toward heated and unheated dye is at present, therefore, not to be explained. But the fact is established.

Therapeutic Significance

It is clear that if this dye possesses any therapeutic value when applied at room temperature one is justified in expecting its potency to be considerably increased by slight increases of temperature. *Not only should the dye be more toxic for Gram-positive organisms when applied hot than when applied cold; it should, when applied hot, become bacteriostatic for certain Gram-negative organisms on which, when applied cold, it is quite without effect.*

And what is true of this dye is probably also true of many other bactericidal agents. The fact should be borne in mind in the extensive studies of this subject now being carried on, in order to determine whether the value of the antiseptics under study may not be considerably increased in this simple way.

As regards gentian violet itself, it is clear that the technique described as having been used with a certain degree of success in the treatment of purulent arthritis *should be modified by the use of hot dye* (after lavage), instead of dye at room temperature.[1]

And the same is true of the use of the dye, by the method of lavage and staining, in the treatment of empyema which will probably follow the work of Watters[2] and of Major,[3] who have demonstrated that the method is feasible and have obtained suggestive results.

It is true that Gay and Morrison[4] have published an experimental study of the treatment of empyema with aniline dyes which leads them to conclude that these substances have little or no future in this condition. But I am inclined to think their conclusions unnecessarily pessimistic. One would hardly expect that the instillation of dye or any other substance into a chest containing a septic effusion, without preliminary mechanical cleansing by aspiration and lavage, would accomplish much, if it accomplished anything. Yet in the majority of the experiments of Gay and Morrison this was the technique employed. Even so, they were able—in many instances—to bring a thorax which had been infected with streptococcus to complete, or almost complete, sterilization. Their conclusion that dyes would never be of value in the treatment of empyema was based on the fact that in these experiments if sterilization was achieved reinfection always occurred. This is exactly what one would have expected if the fact, to which I have so often called attention, were sufficiently appreciated: that these dyes are bacteriostats as well as bactericides. It is not to be expected that every organism, or indeed the majority of organisms, will be reached and killed in a cavity like the chest; but it is not impossible that, if a moderate amount of the dye be kept continually present, growth of organisms would be very difficult, if not impossible.

In attempting to sterilize the sinuses of the head with gentian violet, in human beings, I have had experiences

---

* J. Exp. Med., 1912, XVI, 221.

[1] J. A. M. A., *70:* 1047 (Ap. 13) 1918; *72:* 1280 (May 3) 1919; *74:* 145 (Jan. 17) 1920; *75:* 583 (Aug. 28) 1920; *77:* 24 (July 2) 1921.

[2] Amer. Rev. Tuberculosis, Feb. 1921, No. 12, p. 875.

[3] Amer. Jour. Med. Sc., CLXII, Sept. 21, No. 3, p. 397.

[4] Journ. Inf. Dis., 1921, XXVIII, No. 1, p. 1.

FIG. I.

FIG. II.

FIG. III.

A

FIG. IV.

B

FIG. V.

FIG. VI.

FIG. VII.

similar to those of Gay and Morrison. Even in cases of long standing infection negative cultures may be promptly obtained by mechanical cleansing and instillation of the dye; but re-infection always occurs.

But it is becoming more and more clear that substances which hold bacteria in check, even though they do not kill them, may have real therapeutic value. In localized infections a *sterilisans magna*, though ideal, is not essential. It is well known that in some instances, removal of the great mass of infection from a chest cavity by aspiration has been, alone, sufficient to enable the body to overcome the remaining infection; and it is not unreasonable to hope that if aspiration through a large needle be supplemented by lavage (as I have suggested) and by the instillation of a bacteriostatic agent like gentian violet, a fairly large percentage of these cases may be held in check until the body is able to overcome the infection. At least, the results already obtained with this method, incompletely applied, justify further study along the same lines. For this purpose, the stain should be used hot. It is of course, perfectly clear that dye instilled hot into the body cavities will not remain hot indefinitely, but will, relatively soon, cool to body temperature. We can expect however that during the time when this change in temperature is taking place the potency of the bacteriostatic agent will be greater if the dye has been heated.

FIGURE LEGENDS

FIG. I.—To show that B. subtilis is killed by staining with gentian violet, B. coli unaffected. The organisms have been stroked, after being stained, on plain agar.

S = Unstained subtilis, control.
S′ = Stained subtilis.
C = Unstained B. coli, control.
C′ = Stained B. coli.

FIG. II.—B. coli stroked on plain agar after having been stained with gentian violet at 35°; growth unaffected. Cf. with Fig. III.

FIG. III.—4 strokes have been made of B. coli stained with gentian violet at 50°—no growth. Cf. with Fig. II.

FIG. IV.—Showing that neither heat (50°) *alone*, nor stain *alone* is sufficient to kill B. coli.

A—Stroked with organisms which have been *heated* for 1 hr. to 50°.

B—Stroked with organisms which have been *stained* for 1 hr. at room temperature.

FIG. V.—To show that exposure of B. coli to heat and to gentian violet *in succession* does not kill the organisms. Plate stroked with organisms which have been heated to 50° for 1 hr. and then stained for 1 hr., growth has been unaffected.

FIG. VI.—B. coli which have been stained with gentian violet at room temperature; penetration has been as deep as in those stained with dye at 50°. (See Fig. VII.)

FIG. VII.—B. coli which have been stained with gentian violet at 50°. Cf. Fig. VI.

STUDIES ON EXPERIMENTAL RICKETS. XXI.

AN EXPERIMENTAL DEMONSTRATION OF THE EXISTENCE OF A VITAMIN WHICH PROMOTES CALCIUM DEPOSITION

By E. V. MCCOLLUM, NINA SIMMONDS,
J. ERNESTINE BECKER
(From the Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore)
and
P. G. SHIPLEY
(From the Department of Pediatrics, The Johns Hopkins University, Baltimore)

Although there is no longer any room to doubt the efficacy of cod-liver oil in curing rickets, the nature of the active principle in the oil is still unknown. It has been stated that the fat-soluble A is responsible for the beneficial effect from its administration in this disease. We have, however, published certain evidence which led us to believe that the protection against rickets which cod-liver oil affords is not due to fat-soluble A.

Hopkins has shown that this vitamin is readily destroyed by oxidation. Following his lead we oxidized cod-liver oil for from 12 to 20 hours at 100° C by blowing air through it. Oil which was treated in this way did not cure xerophthalmia, even though its administration was begun at the onset of the disease, when edema of the eyelids was first noticeable. On the other hand, it was just as effective in curing rickets in rats as the untreated oil. The bones of rachitic animals which were given daily a curative dose of the oxidized oil to the amount of 2 per cent of the weight of the ration (average daily consumption of cod-liver oil about 70 mgs.) for 11 days showed quite as advanced evidences of healing as those shown by animals which had received the same amount of unoxidized oil for the same length of time.

The anti-rachitic effect of cod-liver oil is not due, therefore, to its content of fat-soluble A, but to some other factor which the oil contains.

The existence of this substance, which exerts its effects in so remarkable a manner on the growing bones, is now as firmly established as is the existence of any of the three hitherto recognized vitamins. From its mode of action, and the very small amounts of it which are required to exert its directive influence on metabolism, it must, we believe, be classed with this group of essential nutritive principles.

THE JOHNS HOPKINS HOSPITAL REPORTS

VOLUME I. 423 pages, 99 plates.

VOLUME II. 570 pages, with 28 plates and figures.

VOLUME III. 766 pages, with 69 plates and figures.

VOLUME IV. 504 pages, 33 charts and illustrations.

VOLUME V. 480 pages, with 32 charts and illustrations.

VOLUME VI. 414 pages, with 79 plates and figures.

VOLUME VII. 537 pages with illustrations.

VOLUME VIII. 552 pages with illustrations.

VOLUME IX. 1060 pages, 66 plates and 210 other illustrations.
Contributions to the Science of Medicine.

Dedicated by his Pupils to WILLIAM HENRY WELCH, on the twenty-fifth anniversary of his Doctorate. This volume contains 38 separate papers.

VOLUME X. 516 pages, 12 plates and 25 charts.

VOLUME XI. 555 pages, with 38 charts and illustrations.

VOLUME XII. 548 pages, 12 plates and other illustrations.

VOLUME XIII. 605 pages, with 6 plates, 201 figures, and 1 colored chart.

VOLUME XIV. 632 pages, with 97 figures.

VOLUME XV. 542 pages, with 87 illustrations.

Twelve papers on pneumonia. By DRS. CHATARD, FABYAN, EMERSON, MARSHALL, MCCRAE, STEINER, HOWARD and HANES.

A Study of Diarrhœa in Children. J. H. MASON KNOX, JR., M. D., and EDWIN H. SCHOREB, M. D.

Skin Transplantation. By JOHN STAIGE DAVIS, M. D.

Epidemic Cerebrospinal Meningitis and Serum Therapy at The Johns Hopkins Hospital. By FRANK J. SLADEN, M. D.

VOLUME XVI. 670 pages with 151 figures.

Studies in the Experimental Production of Tuberculosis in the Genito-Urinary Organs. By GEORGE WALKER, M. D.

The Effect on Breeding of the Removal of the Prostate Gland or of the Vesiculæ Seminales, or of Both; together with Observations on the Condition of the Testes after such Operations on White Rats. By GEORGE WALKER, M. D.

Scalping Accidents. By JOHN STAIGE DAVIS, M. D.

Obstruction of the Inferior Vena Cava with a Report of Eighteen Cases. By J. HALL PLEASANTS, M. D.

Physiological and Pharmacological Studies on Cardiac Tonicity in Mammals. By PERCIVAL DOUGLAS CAMERON, M. D.

VOLUME XVII. 586 pages with 21 plates and 136 figures.

Free Thrombi and Ball Thrombi in the Heart. By JOSEPH H. HEWITT, M. D.

Benzol as a Leucotoxin. By LAWRENCE SELLING, M. D.

Primary Carcinoma of the Liver. By MILTON C. WINTERNITZ, M. D.

The Statistical Experience Data of The Johns Hopkins Hospital, Baltimore, Md., 1892-1911. By FREDERICK L. HOFFMAN, LL. D., F. S. S.

The Origin and Development of the Lymphatic System. By FLORENCE R. SABIN, M. D.

The Nuclei Tuberis Laterales and the So-called Ganglion Opticum Basale. By EDWARD F. MALONE, M. D.

Venous Thrombosis During Myocardial Insufficiency. By FRANK J. SLADEN, M. D., and MILTON C. WINTERNITZ, M. D.

Leukæmia of the Fowl: Spontaneous and Experimental. By HARRY C. SCHMEISSER, M. D.

VOLUME XVIII. 445 pages with 124 figures.
Fasciculus I.

A Study of a Toxic Substance of the Pancreas. By E. W. GOODPASTURE, M. D., and GEORGE CLARK, M. D.

Old Age in Relation to Cell-overgrowth and Cancer. By E. W. GOODPASTURE, M. D., and G. B. WISLOCKI, M. D.

The Effect of Removal of the Spleen Upon Metabolism in Dogs; Preliminary Report. By J. H. KING, M. D.

The Effect of Removal of the Spleen Upon Blood Transfusion. By J. H. KING, M. D., B. M. BERNHEIM, M. D., and A. T. JONES, M. D.

Studies on Parathyroid Tetany. By D. WRIGHT WILSON, M. D., THORNTON STEARNS, M. D., J. H. JANNEY, JR., M. D., and MADGE DEG. THURLOW, M. D.

Some Observations on the Effect of Feeding Glands of Internal Secretion to Chicks. By M. C. WINTERNITZ, M. D.

Spontaneous and Experimental Leukæmia in the Fowl. By H. C. SCHMEISSER, M. D.

Studies on the Relation of Fowl Typhoid to Leukæmia of the Fowl. By M. C. WINTERNITZ, M. D., and H. C. SCHMEISSER, M. D.

Hyaline Degeneration of the Islands of Langerhans in Pancreatic Diabetes. By M. C. WINTERNITZ, M. D.

Generalized Miliary Tuberculosis Resulting from Extension of a Tubercular Pericarditis Into the Right Auricle. By M. C. WINTERNITZ, M. D.

Acute Suppurative Hypophysitis as a Complication of Purulent Sphenoidal Sinusitis. By T. R. BOGGS, M. D., and M. C. WINTERNITZ, M. D.

A Case of Pulmonary Moniliasis in the United States. By T. R. BOGGS, M. D., and M. C. PINCOFFS, M. D.

Gaucher's Disease (A Report of Two Cases in Infancy). By J. H. M. KNOX, M. D., H. R. WAHL, M. D., and H. C. SCHMEISSER, M. D.

A Fatal Case of Multiple Primary Carcinomata. By E. D. PLASS, M. D.

Congenital Obliteration of the Bile-ducts. By JAMES B. HOLMES, M. D.

Multiple Abscesses of the Brain in Infancy. By JAMES B. HOLMES, M. D.

Gastric Carcinoma in a Woman of Twenty-six Years. By R. G. HUSSEY, M. D.

Subdiaphragmatic Abscess with Rupture Into the Peritoneal Cavity Following Induced Pneumothorax for Pulmonary Hæmorrhage. By R. G. HUSSEY, M. D.

Heart Block Caused by Gumma of the Septum. By E. W. BRIDGEMAN, M. D., and H. C. SCHMEISSER, M. D.

Analysis of Autopsy Records.
A. The Johns Hopkins Hospital. (Table Showing Percentage of Autopsies.)
B. The City Hospitals, Bay View. (Table Showing Percentage of Autopsies.)

"The Monday Conferences."

Clinical Representatives on the Staff of the Department of Pathology.

Donation.

Fasciculus II.

The Role of the Autopsy in the Medicine of To-day. By M. C. WINTERNITZ, M. D.

Experimental Nephropathy in the Dog. Lesions Produced by Injection of *B. bronchisepticus* into the Renal Artery. By M. C. WINTERNITZ, M. D., and WILLIAM C. QUINBY, M. D.

Mesarteritis of the Pulmonary Artery. By M. C. WINTERNITZ, M. D., and H. C. SCHMEISSER, M. D.

A Clinical and Pathological Study of Two Cases of Miliary Tuberculosis of the Choroid. By ROBERT L. RANDOLPH, M. D., and H. C. SCHMEISSER, M. D.

The Blood-vessels of the Heart Valves. By STANHOPE BAYNE-JONES, M. D.

Equilibria in Precipitin Reactions. By STANHOPE BAYNE-JONES, M. D.

Carcinoma of the Pleura with Hypertrophic Osteoarthropathy. Report of a Case with a Description of the Histology of the Bone Lesion. By STANHOPE BAYNE-JONES, M. D.

The Interrelation of the Surviving Heart and Pancreas of the Dog in Sugar Metabolism. By ADMONT H. CLARK, M. D.

Congenital Atresia of the Esophagus with Tracheo-Esophageal Fistula Associated with Fused Kidney. A Case Report and A Summary of the Literature on Congenital Anomalies of the Esophagus. By E. D. PLASS, M. D.

Ectopia Cordis, with a Report of a Case in a Fifteen-Month-Old Infant. By JAMES B. HOLMES, M. D.

Studies in the Mechanism of Absorption from the Colon. By SAMUEL GOLDSCHMIDT, M. D., and A. B. DAYTON, M. D.

Report of Two Fatal Cases Following Percy's Low Heat Treatment of Carcinoma of the Uterus. By V. N. LEONARD, M. D. and A. B. DAYTON, M. D.

The Relationship in Typhoid Between Splenic Infarcts and Peritonitis Unassociated with Intestinal Perforation. By A. B. DAYTON, M. D.

Left Duodenal Hernia. By A. B. DAYTON, M. D.

Histological as Related to Physiological and Chemical Differences in Certain Muscles of the Cat. By H. HAYS BULLARD, M. D.

A Method of Clearing Frozen Sections. By H. HAYS BULLARD, M. D.

On the Occurrence and Significance of Fat in the Muscle Fibers of the Atrio-Ventricular System. By H. HAYS BULLARD, M. D.

Studies on the Metabolism of Cells *in vitro*. 1. The Toxicity of α-Amino-Acids for Embyonic Chicken Cells. By MONTROSE T. BURROWS, M. D., and CLARENCE A. NEYMANN, M. D.

The Significance of the Lunula of the Nail. By MONTROSE T. BURROWS, M. D.

The Oxygen Pressure Necessary for Tissue Activity. By MONTROSE T. BURROWS, M. D.

The Functional Relation of Intercellular Substances in the Body to Certain Structures in the Egg Cell and Unicellular Organisms. By MONTROSE T. BURROWS, M. D.

Studies on the Growth of Cells *in vitro*. The Cultivation of Bladder and Prostrate Tumors Outside the Body. By MONTROSE T. BURROWS, M. D., J. EDWARD BURNS, M. D., and YOSHIO SUZUKI, M. D.

The Study of a Small Outbreak of Poliomyelitis in an Apartment House Occurring in the Course of an Epidemic in a Large City. By MONTROSE T. BURROWS, M. D., and EDWARDS A. PARK, M. D.

Papilloma of the Larynx. Report of a Case Treated with Radium with Resultant Chronic Diffuse Thyroiditis. By WILLIAM C. DUFFY, M. D.

Analysis of Autopsy Records.

Autopsy Statistics.
(a) Bay View.
(b) Johns Hopkins Hospital.

Report of the Photographic Department.

General Improvements.

Donations.

VOLUME XIX. 358 pages with 29 plates.

The Structure of the Normal Fibers of Purkinje in the Adult Human Heart and Their Pathological Alteration in Syphilitic Myocarditis. By O. VAN DER STRICHT and T. WINGATE TODD, M. D.

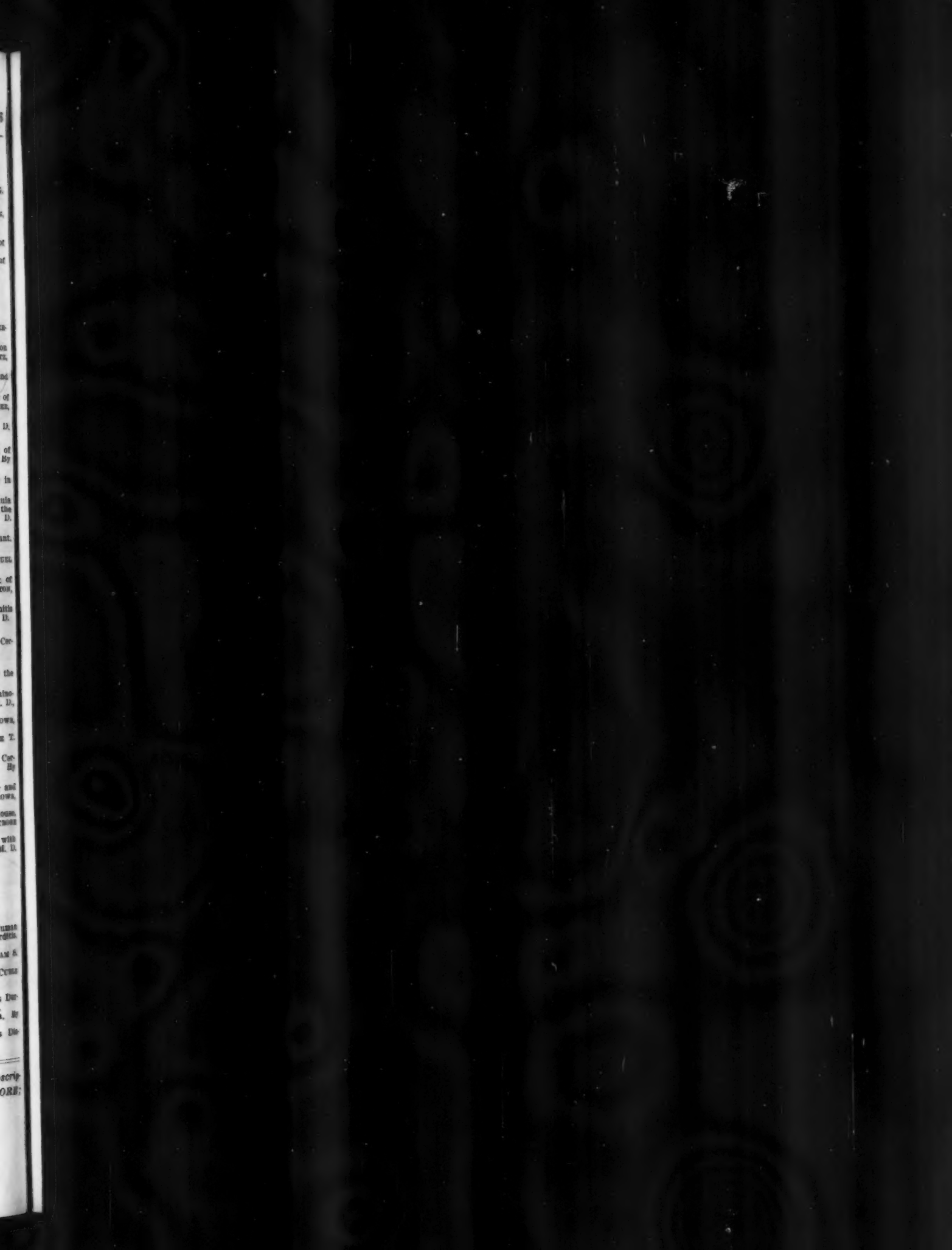
The Operative Story of Goitre. The Author's Operation. By WILLIAM S. HALSTED, M. D.

Study of Arterio-Venous Fistula with an Analysis of 447 Cases. By CARL L. CALLANDER, M. D.

VOLUME XX. 314 pages with 82 plates.

The Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917-18. By WILLIAM G. MACCALLUM, M. D.

Pathological Anatomy of Pneumonia Associated with Influenza. By WILLIAM G. MACCALLUM, M. D.

Lymphosarcoma. Lymphatic Leukæmia. Leucosarcoma. Hodgkin's Disease. LESLIE T. WEBSTER, M. D.

The Johns Hopkins Hospital Bulletins are issued monthly. They are printed by MEYER & THALHEIMER, Baltimore. Subscriptions, $4.00 a year (foreign postage, 50 cents), may be addressed to the publishers, THE JOHNS HOPKINS PRESS, BALTIMORE; single copies will be sent by mail for fifty cents each.